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(54) Title: CANINE CYCLOOXYGENASE-1 (COX-1) AND CYCLOOXYGENASE-2 (COX-2)

(57) Abstract: The present invention relates to genes that encode proteins of the canine cyclooxygenase-1 (COX-1) and the cyclooxygenase-2 (COX-2) enzymes, the canine COX-1 and canine COX-2 proteins, and assays for the evaluation of canine COX-1 and COX-2 activities. The invention also relates to nucleic acid molecules associated with these genes including their complements, homologues and fragments, and to methods of using these nucleic acid molecules, the enzymes and fragments of either.

CANINE CYCLOOXYGENASE -1 (COX-1) AND CYCLOOXYGENASE-2 (COX-2)

Field of the Invention

The present invention relates to genes that encode proteins of canine cyclooxygenase-1 (COX-1) and canine cyclooxygenase-2 (COX-2) enzymes, to canine COX-1 and canine COX-2 proteins, and to assays for the evaluation of canine COX-1 and COX-2 activities. The invention also relates to nucleic acid molecules associated with these genes and their complements, homologues and fragments, and to methods of using these nucleic acid molecules, the enzymes and fragments of either.

Background

Millions of dogs are kept either as pets or for utilitarian uses (e.g., seeing-eye dogs or guard dogs) (Olson and Moulton, *J Reprod Fertil Suppl* 47:433-8 (1993)). It is well known that chronic inflammatory processes such as degenerative joint disease are common in canine species, ie. dogs, especially older dogs. There are, however, few drugs that can be successfully used in veterinary medicine for the treatment of inflammation (*Compendium of Veterinary Products*, Bennett 2d ed., 1993). Therefore, there is a large ongoing effort to find pharmaceutical agents which will impede or altogether stop the progress of such inflammatory disease processes in dogs, or at least ameliorate the symptoms of the inflammation such as pain and edema.

Non-steroidal anti-inflammatory drugs, or NSAIDs, have been investigated extensively for anti-inflammatory activity in humans and in a more limited manner, in dogs. Only two NSAIDs have been approved for use in dogs by the Food and Drug Administration, Committee on Veterinary Medicine (FDA/CVM), and this has resulted in substantial off-label use in dogs of NSAIDs approved for humans.

NSAID use in dogs has led to severe injury and even death due to gastrointestinal hemorrhage, perforation and peritonitis, and kidney and liver toxicity. Although NSAID toxicities are also seen in humans, they are more pronounced in dogs due to inappropriately high doses from a lack of dosing information, as well as the inherently high degree of canine susceptibility to such adverse reactions. An alternative to NSAIDs, corticosteroids, has even more drastic side effects, especially when long term therapy is involved (MacDonald and Langston, Use of Corticosteroids and Non-steroidal Anti-inflammatory Agents, in: *Textbook of Veterinary Internal Medicine*, 284, Ettinger and Feldman, ed. (1995)).

The toxicity of NSAIDs is a result of their lack of specificity in prostaglandin inhibition. Prostaglandins, a group of compounds derived from arachidonic acid by the action of cyclooxygenase, are known to be involved in the inflammatory process. Some of these prostaglandins mediate inflammation; however, some of these prostaglandins also have a gastrointestinal protective effect. Previous NSAIDs were found to prevent prostaglandin release by inhibiting the enzyme cyclooxygenase (COX) (Rubin and Papich, *Curr. Vet. Ther.* X:47-54 (1989)). The recent discovery of an inducible cyclooxygenase enzyme associated with the inflammation (cyclooxygenase II or COX-2), provides a viable target of inhibition which can effectively reduce inflammation and produce fewer and less drastic side effects than occurs by targeting both COX-1 and COX-2 enzymes. It has been shown that the COX-2 isozyme is responsible for the production of prostaglandins exclusively or primarily at inflammatory sites. Drugs that selectively inhibit the human COX-2 isozyme relative to the human COX-1 isozyme can reduce inflammation without the side effects of gastrointestinal toxicity in humans.

For many years, the only known cyclooxygenase was the COX-1 isoform. Since its discovery, the inducible form (COX-2) has been cloned, sequenced, and characterized from chicken, sheep, murine, and human sources. These purified

enzymes have provided a means for assays and materials to identify and evaluate human pharmaceutical agents that selectively inhibit COX-2 relative to COX-1.

Summary of the Invention

A substantially-purified canine form of the COX-1 and COX-2 enzymes would provide a means to carry out assays and materials valuable in identifying drugs that can reduce inflammation in dogs and which are without the toxic effects of current canine therapies. Thus, there is a substantial need in the art for the cloned, sequenced and characterized canine COX-1 and COX-2, and assays to screen and evaluate selective inhibitors of COX-2.

The present invention provides a substantially-purified nucleic acid molecule which specifically hybridizes to a nucleic acid molecule having a SEQ ID described herein or complement thereof, wherein the substantially-purified nucleic acid molecule encodes for a canine COX-2 protein or fragment thereof or complement of either. The present invention also provides a substantially-purified nucleic acid molecule which specifically hybridizes to a SEQ ID described herein or complement thereof, wherein the substantially-purified nucleic acid molecule encodes for a canine COX-1 protein or fragment thereof or complement of either. Substantially-purified protein sequences of canine COX-1 and COX-2 are also disclosed. This invention also provides assays and materials to identify and evaluate veterinary agents that are potent inhibitors of canine COX-2.

In one embodiment the present invention provides a substantially-purified nucleic acid molecule selected from the group of DNAs consisting of a nucleic acid molecule having SEQ ID: 1, the complement of the nucleic acid molecule having SEQ ID:1, an internal fragment of the nucleic acid molecule having SEQ ID: 1, and an internal fragment of the complement of the nucleic acid molecule having SEQ ID: 1, wherein SEQ ID: 1 encodes a canine COX-1 protein or a fragment thereof.

In another embodiment the present invention provides a substantially-purified canine COX-1 nucleic acid molecule which comprises a nucleic acid sequence that is identical to at least 20 contiguous nucleotides of SEQ ID: 1 or a complement thereof.

In another embodiment the present invention provides a substantially-purified canine COX-1 protein or a fragment thereof encoded by a nucleic acid sequence which specifically hybridizes to SEQ ID: 1 or a complement thereof.

In another embodiment the present invention provides a substantially-purified canine COX-1 protein having SEQ ID: 2.

In another embodiment the present invention provides a substantially-purified canine COX-1 protein or a fragment thereof comprising at least 10 consecutive amino acids of SEQ ID: 2.

In another embodiment the present invention provides an antibody capable of specifically binding to a substantially-purified canine COX-1 protein having SEQ ID: 2.

In another embodiment the present invention provides a substantially-purified nucleic acid molecule selected from the group of DNAs consisting of a nucleic acid molecule having SEQ ID: 3, a complement molecule of a nucleic acid molecule having SEQ ID: 3, an internal fragment a nucleic acid molecule having SEQ ID: 3, and an internal fragment of a complement molecule of a nucleic acid molecule having SEQ ID: 3, wherein the SEQ ID: 3 encodes a canine COX-2 protein or a fragment thereof.

In another embodiment the present invention provides a substantially-purified canine COX-2 nucleic acid molecule which comprises a nucleic acid sequence that is identical to at least 20 contiguous nucleotides of SEQ ID: 3 or its complement.

In another embodiment the present invention provides a substantially-purified canine COX-2 protein or fragment thereof encoded by a nucleic acid sequence which specifically hybridizes to SEQ ID: 4 or its complement.

In another embodiment the present invention provides a substantially-purified canine COX-2 protein having SEQ ID: 4.

In another embodiment the present invention provides a substantially-purified canine COX-2 protein or a fragment thereof wherein the protein or the fragment thereof comprises at least 10 consecutive amino acids of SEQ ID: 4.

In another embodiment the present invention provides a antibody capable of specifically binding to the substantially-purified canine COX-2 protein having SEQ ID: 4.

In another embodiment the present invention provides a transformed cell having a nucleic acid molecule which comprises an exogenous promoter region linked to a structural nucleic acid sequence, and a 3'non-translated sequence linked to the structural nucleic acid sequence, wherein the exogenous promoter region causes the production of a mRNA molecule; the structural nucleic acid sequence encodes a canine COX-1 gene or a fragment thereof; and the 3' non-translated sequence causes termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of the mRNA molecule.

In another embodiment the present invention provides a transformed cell having a nucleic acid molecule which comprises an exogenous promoter region linked to a structural nucleic acid sequence, and a a 3' non-translated sequence, wherein the exogenous promoter region causes the production of a mRNA molecule; the structural nucleic acid molecule encodes a canine COX-2 gene or a fragment thereof; and the 3' non-translated sequence causes termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of the mRNA molecule.

In another embodiment the present invention provides a method for assaying a canine COX-2 protein in a cell comprising (A) incubating a marker nucleic acid molecule with a complementary nucleic acid molecule obtained from the cell, wherein the incubation is performed under conditions permitting nucleic acid hybridization, and the marker nucleic acid molecule is capable of specifically hybridizing under high

stringency conditions to a canine COX-2-encoding nucleic acid molecule or a complement thereof; (B) hybridizing the marker nucleic acid molecule with the complementary nucleic acid molecule; and (C) assaying the complementary nucleic acid, wherein the assay is predictive of the level or pattern of the canine COX-2 protein.

In another embodiment the present invention provides a method for diagnosis or prognosis of a COX-2 related condition in a dog wherein the method comprises assaying the concentration of a molecule, the concentration being dependent upon the expression of a canine COX-2 gene, and the molecule being assayable in a biological sample from the dog.

In another embodiment the present invention provides a method for diagnosing a COX-2 related condition in a dog which comprises the steps of: (A) incubating under conditions permitting a nucleic acid hybridization a marker nucleic acid molecule with a complementary nucleic acid molecule obtained from a biological sample from the dog; (B) hybridizing the marker nucleic acid molecule with the complementary nucleic acid molecule; and (C) assaying the hybridized marker nucleic acid molecule and complementary nucleic acid molecule for a polymorphism; thereby diagnosing the COX-2 related condition in the dog wherein: the marker nucleic acid molecule comprises a nucleotide sequence of a polynucleotide that specifically hybridizes to another polynucleotide that is linked to a canine COX-2 gene; and the polymorphism is predictive of a mutation affecting the canine COX-2 condition in the dog.

In another embodiment the present invention provides a method of determining an association between a polymorphism and a trait expressed by a dog wherein the method comprises: (A) hybridizing a nucleic acid molecule specific for the polymorphism to a gene in a cell, wherein the nucleic acid molecule has a nucleic acid sequence of SEQ ID: 3 or a complement thereof; and (B) determining the degree of association between the polymorphism and the trait.

In another embodiment the present invention provides a method of producing a cell containing an expressed canine COX-2 protein comprising: (A) transforming the cell with a functional nucleic acid molecule; and (B) culturing the transformed

cell; thereby producing the cell containing the expressed canine COX-2 protein, wherein: the functional nucleic acid molecule comprises a promoter region, the promoter region is linked to a structural region, the structural region comprises a nucleic acid sequence of SEQ ID: 3, the structural region is linked to a 3' non-translated sequence that functions in the cell to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of a mRNA molecule; and the functional nucleic acid molecule results in expression of the canine COX-2 protein.

In another embodiment the present invention provides a method of producing a cell exhibiting co-suppression of a canine COX-2 protein comprising: (A) transforming the cell with a functional nucleic acid molecule; and (B) culturing the transformed cell; thereby producing the cell exhibiting co-suppression of the canine COX-2 protein, wherein: the functional nucleic acid molecule comprises a promoter region, the promoter region is linked to a structural region, the structural region comprises a nucleic acid sequence of SEQ ID: 3, the structural region is linked to a 3' non-translated sequence that functions in the cell to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of a mRNA molecule, and the functional nucleic acid molecule results in co-suppression of the canine COX-2 protein.

In another embodiment the present invention provides a method for causing co-suppression of a canine COX-2 protein in a transformed cell comprising: (A) transforming the cell with a nucleic acid molecule to produce a transformed cell; and (B) culturing the transformed cell; thereby causing co-suppression of the canine COX-2 protein in the transformed cell, wherein: the functional nucleic acid molecule comprises a promoter region; the promoter region is linked to a structural region, the structural region comprises a nucleic acid molecule having a nucleic acid sequence of SEQ ID: 3, the structural region is linked to a 3' non-translated sequence that functions in the cell to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of a mRNA molecule, and the functional nucleic acid molecule results in co-suppression of the canine COX-2 protein.

In another embodiment the present invention provides a method of isolating a nucleic acid molecule that encodes a canine COX-2 protein or a fragment thereof

comprising (A) incubating under conditions permitting nucleic acid hybridization a first nucleic acid molecule comprising SEQ ID: 3 or the complement thereof with a complementary second nucleic acid molecule obtained from a cell; (B) hybridizing the first nucleic acid molecule with the second nucleic acid molecule; and (C) isolating the second nucleic acid molecule.

In another embodiment the present invention provides a method of producing a cell containing an expressed canine COX-2 gene comprising (A) transforming the cell with a functional nucleic acid molecule; and (B) culturing the transformed cell; thereby producing the cell containing the expressed canine COX-2 gene, wherein: the functional nucleic acid molecule comprises a promoter region, the promoter region is linked to a structural region wherein the structural region comprises a nucleic acid molecule having a nucleic acid sequence consisting of SEQ ID:3 or complements thereof the structural region is linked to a 3' non-translated sequence that functions in the cell to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of a mRNA molecule, and the functional nucleic acid molecule results in overexpression of the canine COX-2 gene protein.

In another embodiment the present invention provides a method of producing a cell exhibiting co-suppression of a canine COX-2 gene comprising (A) transforming the cell with a functional nucleic acid molecule; and (B) culturing the transformed cell; thereby producing a cell co-suppression of a canine COX-2 gene, wherein: the functional nucleic acid molecule comprises a promoter region, the promoter region is linked to a structural region wherein the structural region comprises a nucleic acid sequence selected from the group consisting of SEQ ID: 3 or complements thereof, the structural region is linked to a 3' non-translated sequence that functions in the cell to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of a mRNA molecule, and the functional nucleic acid molecule results in co-suppression of the canine COX-2 gene.

In another embodiment the present invention provides a method for causing co-suppression of a canine COX-2 gene in a cell comprising (A) transforming the cell with a nucleic acid molecule; and (B) culturing the transformed cell; thereby reducing expression of a canine COX-2 gene in a cell, wherein the nucleic acid molecule has an

exogenous promoter region which causes the production of a mRNA molecule, the exogenous promoter region is linked to a transcribed nucleic acid molecule having a transcribed strand and a non-transcribed strand, the transcribed strand is complementary to a nucleic acid molecule having a nucleic acid selected from the group consisting of SEQ ID: 3 or complements thereof and the transcribed strand is complementary to an endogenous mRNA molecule, and the transcribed nucleic acid molecule is linked to a 3' non-translated sequence that functions in the cell to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of a mRNA molecule.

In another embodiment the present invention provides a substantially-purified nucleic acid molecule comprising a sequence which encodes a mammalian canine COX-2 protein.

In another embodiment the present invention provides a method of identifying a test material which has the ability to inhibit, suppress, modulate, or maintain canine COX-2 activity wherein the method comprises (A) contacting a test transformed cell with the test material, wherein the transformed cell expresses a gene encoding canine COX-2, and wherein the contacting is performed in the presence of test amount of prostaglandin precursor; (B) measuring the conversion of prostaglandin precursor to prostaglandin product by the test transformed cells; (C) contacting a control transformed cell with a control amount of prostaglandin precursor, wherein the control transformed cell expresses a gene encoding canine COX-2, and wherein the contacting is performed substantially in the absence of the test material; (D) measuring the conversion of prostaglandin precursor to prostaglandin product by the control transformed cells; and (E) comparing the amount of prostaglandin precursor converted to prostaglandin product by the test transformed cells to the amount of prostaglandin precursor converted to prostaglandin product by the control transformed cells. In a preferred embodiment, the prostaglandin precursor is arachadonic acid.

Brief Description of the Drawings

Fig. 1 diagramatically sets forth a comparison between the amino acid sequence which codes for canine COX-1 and the amino acid sequence which codes for human COX-1.

Fig. 2 diagramatically sets forth a comparison between the amino acid sequences of canine COX-2 and human COX-2.

Fig. 3 diagramatically sets forth a comparison between the amino acid sequences of murine, rat, guineapig, rabbit, horse, canine, sheep, human and chicken COX-2.

SEQ NO: 1 – canine (*Canis familiaris*) COX-1 nucleic acid sequence

SEQ NO: 2 – canine (*Canis familiaris*) COX-1 amino acid sequence

SEQ NO: 3 – canine (*Canis familiaris*) COX-2 nucleic acid sequence

SEQ NO: 4 – canine (*Canis familiaris*) COX-2 amino acid sequence

SEQ NO: 5 – human (*Homo sapiens*) COX-1 nucleic acid sequence

SEQ NO: 6 – human (*Homo sapiens*) COX-1 amino acid sequence

SEQ NO: 7 – human (*Homo sapiens*) COX-2 nucleic acid sequence

SEQ NO: 8 - human (*Homo sapiens*) COX-2 amino acid sequence

SEQ NO: 9 - mouse (*Mus muscus*) COX-2 amino acid sequence

SEQ NO: 10 - rat (*Rattus norvegicus*) COX-2 amino acid sequence

SEQ NO: 11 - guinea pig (*Cavia porcellus*) COX-2 amino acid sequence

SEQ NO: 12 - rabbit (*Oryctolagus cuniculus*) COX-2 amino acid sequence

SEQ NO: 13 - horse (*Equus caballus*) COX-2 amino acid sequence

SEQ NO: 14 - sheep (*Ovis aries*) COX-2 amino acid sequence

SEQ NO: 15 - chicken (*Gallus gallus*) COX-2 amino acid sequence

These detailed descriptions are presented for illustrative purposes only and are not intended as a restriction on the scope of the invention. Rather, they are merely some of the embodiments that one skilled in the art would understand given the entire contents of this disclosure. All parts are by weight and temperatures are in Degrees centigrade unless otherwise indicated.

The following is a list of abbreviations and the corresponding meanings as used interchangeably herein:

IMDM = Iscove's modified Dulbecco's media

mg = milligram

ml = milliliter

mL = milliliter

µg = microgram

µl = microliter

ODNs= oligonucleotides

PCR= polymerase chain reaction

RP-HPLC = reverse phase high performance liquid chromatography

ug = microgram

ul = microliter

The following is a list of definitions of various terms used herein:

The term "altered" means that expression differs from the expression response of cells or tissues not exhibiting the phenotype.

The term "amino acid(s)" means all naturally occurring L-amino acids.

The term "chromosome walking" means a process of extending a genetic map by successive hybridization steps.

The term "complete complementarity" means that every nucleotide of one molecule is complementary to a nucleotide of another molecule.

The term "degenerate" means that two nucleic acid molecules encode for the same amino acid sequences but comprise different nucleotide sequences.

The term "exogenous genetic material" means any genetic material, whether naturally occurring or otherwise, from any source that is capable of being inserted into any organism..

The term "expression response" means the mutation affecting the level or pattern of the expression encoded in part or whole by one or more nucleic acid molecules.

The term "fragment" means a nucleic acid molecule whose sequence is shorter than the target or identified nucleic acid molecule and having the identical, the substantial complement, or the substantial homologue of at least 10 contiguous nucleotides of the target or identified nucleic acid molecule.

The term "fusion molecule" means a protein-encoding molecule or fragment that upon expression, produces a fusion protein.

The term "fusion protein" means a protein or fragment thereof that comprises one or more additional peptide regions not derived from that protein.

The term "marker nucleic acid" means a nucleic acid molecule that is utilized to determine an attribute or feature (*e.g.,* presence or absence, location, correlation, etc.) of a molecule, cell, or tissue.

The term "mimetic" refers to a compound having similar functional and/or structural properties to another known compound or a particular fragment of that known compound.

The term "phenotype" means any of one or more characteristics of an organism, tissue, or cell.

The term "probe" means an agent that is utilized to determine an attribute or feature (e.g. presence or absence, location, correlation, etc.) of a molecule, cell, tissue, or organism.

The term "promoter" is used in an expansive sense to refer to the regulatory sequence(s) that control mRNA production.

The term "protein fragment" means a peptide or polypeptide molecule whose amino acid sequence comprises a subset of the amino acid sequence of that protein.

The term "protein molecule" or "peptide molecule" means any molecule that comprises five or more amino acids.

The term "recombinant" means any agent (e.g., DNA, peptide, etc.), that is, or results from, however indirectly, human manipulation of a nucleic acid molecule.

The term "selectable or screenable marker genes" means genes who's expression can be detected by a probe as a means of identifying or selecting for transformed cells.

The term "specifically bind" means that the binding of an antibody or peptide is not competitively inhibited by the presence of non-related molecules.

The term "specifically hybridizing" means the quality of having a very high level of affinity for another material, for example, of one nucleic acid strand for a complementary strand having a high degree of homology.

The term "structural nucleic acid sequence" means a nucleic acid sequence that carries the coding for the amino acid sequence of a specific protein.

The term "substantial complement" means that a nucleic acid sequence shares at least 80% sequence identity with the complement.

The term "substantial fragment" means a fragment which comprises at least 100 nucleotides.

The term "substantial homologue" means that a nucleic acid molecule shares at least 80% sequence identity with another.

The term "substantially hybridizing" means that two nucleic acid molecules can form an anti-parallel, double-stranded nucleic acid structure under conditions (e.g. salt and temperature) that permit hybridization of sequences that exhibit 90% sequence identity or greater with each other and exhibit this identity for at least a contiguous 50 nucleotides of the nucleic acid molecules.

The term "substantially purified" means that one or more molecules that are or may be present in a naturally occurring preparation containing the target molecule will have been removed or reduced in concentration.

The term "trait" means any genetically determined condition or state including a healthy condition, a disease condition, or a passive condition.

Detailed Description of the Invention

I. Overview

The present invention stems in part from the isolation of cDNA molecules that encode canine COX-1 and canine COX-2. SEQ ID: 1 sets forth the nucleic acid sequence of canine COX-1 and SEQ ID: 2 sets forth the amino acid sequence of canine COX-1. SEQ ID: 3 sets forth the nucleic acid sequence of canine COX-2 and SEQ ID: 4 sets forth the amino acid sequence of canine COX-2. Figure 1 diagramatically sets forth a comparison between the amino acid sequences of human COX-1 and canine COX-1. Figure 2 diagramatically sets forth a comparison between the amino acid sequence which codes for human COX-2 and the amino acid sequence which codes for canine COX-2. Figure 3 diagramatically sets forth a comparison between the nucleic acid sequences of human and canine COX-2, and their consensus sequence. Figure 4 sets forth a multiple sequence alignment of the cyclooxygenase-2 isozymes from mouse, rat, guinea pit, rabbit, horse, canine, sheep, human, and chicken.

II. Molecules f the Present Invention

The methods of the present invention are particularly useful in the monitoring of the expression of canine COX-2. The methods of the present invention are further useful in screening materials, compounds or compositions that selectively inhibit canine COX-2. The methods of the present invention are also relevant in the monitoring of the expression of canine COX-2 in canines with COX-2 related conditions, especially inflammatory joint disease and cancer. Molecules of the present invention are capable of being used to diagnose abnormal canine COX-2 expression. Molecules of the present invention can also be used as therapeutic agents. The molecules of the present invention may be either naturally occurring or non-naturally occurring (i.e., synthetically prepared). As used herein, a naturally occurring molecule may be "substantially-purified," if desired, such that one or more impurities that are or may be present in a naturally-occurring preparation containing the molecule will have been removed or will be present at a lower concentration than that at which they would normally be found.

The molecules of the present invention will preferably be "biologically active" with respect to either a structural attribute, such as the capacity of one nucleic acid molecule to hybridize to another nucleic acid molecule, or the ability of a protein molecule to be bound by an antibody molecule (or to compete with another molecule for such binding). Alternatively, such an attribute may be catalytic, and thus involve the capacity of the agent to mediate a chemical reaction or response.

The molecules of the present invention comprise nucleic acid molecules, proteins, and small organic compounds (i.e., having a molecular weight of less than about 2,000).

A. Nucleic Acid Molecules

A preferred class of agents of the present invention comprises canine COX-2 nucleic acid molecules. Such molecules may be either DNA or RNA.

In one embodiment, such nucleic acid molecules will encode all or a fragment of canine COX-2 protein, its "promoter," or its flanking gene sequences. The term "promoter" is used herein in an expansive sense to refer to the regulatory sequence(s) that control mRNA production. Such sequences include RNA polymerase binding sites, enhancers, etc. All such canine COX-2 molecules may be used in a diagnostic or therapeutic context.

Fragment canine COX-2 nucleic acid molecules may encode significant portion(s) of, or indeed most of, the canine COX-2 protein. Preferably, a fragment canine COX-2 nucleic acid molecule is identical or complementary to at least 20 contiguous nucleotides in SEQ ID: 3. More preferably it comprises at least 25 nucleotides in SEQ ID: 3. Even more preferably it comprises at least 50 nucleotides in SEQ ID: 3. More preferably still, it comprises at least 100 nucleotides in SEQ ID: 3. In a preferred embodiment, a fragment canine COX-2 nucleic acid molecule comprises at least one nucleotide that is not found in a corresponding position in canine COX-2.

Canine COX-2 nucleic acid molecules and fragment canine COX-2 nucleic acid molecules can specifically or selectively hybridize with other nucleic acid molecules. As used herein, two nucleic acid molecules are said to be capable of specifically hybridizing to one another when they have a very high level of affinity for one another, such as when one nucleic acid strand has a high degree of homology to a complementary strand. A nucleic acid molecule is the to be the "complement" of another nucleic acid molecule if they exhibit complete complementarity. As used herein, molecules are the to exhibit "complete complementarity" when every nucleotide of one of the molecules is complementary to a nucleotide of the other. Two molecules are the to be "minimally complementary" if they can hybridize to one another with sufficient stability to permit them to remain annealed to one another under at least conventional "low-stringency" conditions. Similarly, the molecules are the to be "complementary" if they can hybridize to one another with sufficient

stability to permit them to remain annealed to one another under conventional "high-stringency" conditions. Conventional stringency conditions are described by Sambrook, *et al.*, *Molecular Cloning*, A Laboratory Manual, 2nd Ed., Cold Spring Harbor Press, Cold Spring Harbor, New York (1989). Conventional stringency conditions are further described by Haymes, *et al. Nucleic Acid Hybridization, A Practical Approach*, IRL Press, Washington, DC (1985). Departures from complete complementarity are therefore permissible, as long as such departures do not completely preclude the capacity of the molecules to form a double-stranded structure.

In another preferred embodiment, the fragment canine COX-2 molecule exhibits canine COX-2 enzymatic activity.

In one embodiment the present invention provides a substantially-purified nucleic acid molecule selected from the group of DNAs consisting of a nucleic acid molecule having SEQ ID: 1, the complement of the nucleic acid molecule having SEQ ID:1, an internal fragment of the nucleic acid molecule having SEQ ID: 1, and an internal fragment of the complement of the nucleic acid molecule having SEQ ID: 1, wherein SEQ ID: 1 encodes a canine COX-1 protein or a fragment thereof. Preferably, the the substantially-purified nucleic acid molecule encodes a canine COX-1 protein. Also preferably, the substantially-purified nucleic acid molecule has SEQ ID: 1 or a complement thereof. In another embodiment of the present invention the substantially-purified nucleic acid molecule specifically hybridizes to another nucleic acid molecule that encodes canine COX-1 or complement thereof and fails to specifically hybridize to a nucleic acid molecule that encodes non-canine COX-1 or complement thereof. In yet another embodiment, the substantially-purified nucleic acid molecule according to claim 4, wherein the nucleic acid molecule specifically hybridizes to another nucleic acid molecule that encodes canine COX-1 or complement thereof under high stringency conditions and fails to specifically hybridize to a nucleic acid molecule that encodes non-canine COX-1 or complement

thereof under high stringency conditions. In one preferred embodiment, the internal fragment of the nucleic acid molecule having SEQ ID: 1 contains at least 50 nucleic acid residues, more preferably at least 100 nucleic acid residues, more preferably still at least 200 nucleic acid residues, and still more preferably at least 500 nucleic acid residues.. In another preferred embodiment, the internal fragment of the complement of the nucleic acid molecuel having SEQ ID: 1 contains at least 50 nucleic acid residues, more preferably at least 100 nucleic acid residues, more preferably still at least 200 nucleic acid residues, and still more preferably at least 500 nucleic acid residues.

In another embodiment, the present invention provides a substantially-purified canine COX-1 nucleic acid molecule which comprises a nucleic acid sequence that is identical to at least 20 contiguous nucleotides of SEQ ID: 1 or the complement of SEQ ID: 1. Preferably, the nucleic acid molecule comprises a nucleic acid sequence that is identical to at least 50 contiguous nucleotides of SEQ ID: 1 or a complement thereof. More preferably, the nucleic acid molecule comprises a nucleic acid sequence that is identical to at least 100 contiguous nucleotides of SEQ ID: 1 or a complement thereof.

The present invention also provides a substantially-purified nucleic acid molecule selected from the group of DNAs consisting of a nucleic acid molecule having SEQ ID: 3, a complement molecule of a nucleic acid molecule having SEQ ID: 3, an internal fragment a nucleic acid molecule having SEQ ID: 3, and an internal fragment of a complement molecule of a nucleic acid molecule having SEQ ID: 3, wherein the SEQ ID: 3 encodes a canine COX-2 protein or a fragment thereof. Preferably the substantially-purified nucleic acid molecule encodes the canine COX-2 protein. More preferably the substantially-purified nucleic acid molecule has the sequence described by SEQ ID: 3 or a complement thereof. In another embodiment the present invention provides a substantially-purified nucleic acid molecule specifically hybridizes to another nucleic acid molecule that encodes canine COX-2 or

complement thereof and fails to specifically hybridize to a nucleic acid molecule that encodes non-canine COX-2 or complement thereof. Preferably, the substantially-purified nucleic acid molecule specifically hybridizes to another nucleic acid molecule that encodes canine COX-2 or complement thereof under high stringency conditions and fails to specifically hybridize to a nucleic acid molecule that encodes non-canine COX-2 or complement thereof under high stringency conditions. In one preferred embodiment, the internal fragment of the nucleic acid molecule having SEQ ID: 3 contains at least 50 nucleic acid residues, more preferably at least 100 nucleic acid residues, more preferably still at least 200 nucleic acid residues, and still more preferably at least 500 nucleic acid residues. In another preferred embodiment, the internal fragment of the complement of the nucleic acid molecule having SEQ ID: 3 contains at least 50 nucleic acid residues, more preferably at least 100 nucleic acid residues, more preferably still at least 200 nucleic acid residues, and still more preferably at least 500 nucleic acid residues.

The present invention further provides a substantially-purified canine COX-2 nucleic acid molecule which comprises a nucleic acid sequence that is identical to at least 20 contiguous nucleotides of SEQ ID: 3 or a complement thereof. Preferably, the nucleic acid sequence that is identical to at least 50 contiguous nucleotides of SEQ ID: 3 or a complement thereof. More preferably the nucleic acid molecule comprises a nucleic acid sequence that is identical to at least 100 contiguous nucleotides of SEQ ID: 3 or its complement.In general terms, stringency conditions which promote DNA hybridization are, for example, 6.0 x sodium chloride/sodium citrate (SSC) at about 45°C, followed by a wash of 2.0 x SSC at 50°C, are known to those skilled in the art or can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. For example, the salt concentration in the wash step can be selected from a low stringency of about 2.0 x SSC at 50°C to a high stringency of about 0.2 x SSC at 50°C. In addition, the temperature in the wash step can be increased from low stringency conditions at room temperature, about 22°C, to high

stringency conditions at about 65°C. Both temperature and salt may be varied, or either the temperature or the salt concentration may be held constant while the other variable is changed.

In a preferred embodiment, a nucleic acid of the present invention will specifically hybridize to a nucleic acid molecule having SEQ ID: 3 or its complement under moderately stringent conditions, for example at about 2.0 x SSC and about 40°C.

In a particularly preferred embodiment, a nucleic acid of the present invention will specifically hybridize to SEQ ID: 3 or its complement under high stringency conditions. In one aspect of the present invention, a nucleic acid molecule of the present invention will comprise SEQ ID: 3 or its complement.

Fragment nucleic acid molecules can be determined and selected such that under specified conditions, such as high stringency, the fragment nucleic acid molecules can be used to specifically hybridize to canine COX-2 relative, for example, to canine COX-1.

In another aspect of the present invention, a nucleic acid molecule of the present invention shares between 100% and 90% sequence identity with the nucleic acid sequence set forth in SEQ ID: 3 or its complement. In a preferred aspect of the present invention, a nucleic acid molecule of the present invention shares between 100% and 95% sequence identity with the nucleic acid sequence set forth in SEQ ID: 3 or its complement. In a more preferred aspect of the present invention, a nucleic acid molecule of the present invention shares between 100% and 98% sequence identity with SEQ ID: 3 or its complement. In the most preferred aspect of the present invention, a nucleic acid molecule of the present invention shares between 100% and 99% sequence identity with SEQ ID: 3 or its complement.

The degeneracy of the genetic code, which allows different nucleic acid sequences to code for the same protein or peptide, is known in the literature. (U.S. Patent No. 4,757,006). As used herein, a nucleic acid molecule is degenerate of

another nucleic acid molecule when the nucleic acid molecules encode for the same amino acid sequences, but comprise different nucleotide sequences. An aspect of the present invention is that the nucleic acid molecules of the present invention include nucleic acid molecules that are degenerate of SEQ ID: 3 and its complement.

Apart from their other uses such as those described below, the nucleic acid molecules of the present invention can be employed to obtain other canine COX-2 molecules. Nucleic acid molecules and fragments thereof of the present invention may be employed to obtain other nucleic acid molecules from dogs. Such nucleic acid molecules include the nucleic acid molecules that encode the complete coding sequence of a protein and promoters and flanking sequences of such molecules. In addition, such nucleic acid molecules include nucleic acid molecules that encode for other isozymes or gene family members. Such molecules can be readily obtained by using the above-described nucleic acid molecules, or fragments thereof, to screen cDNA or genomic libraries obtained from species. Methods for forming such libraries are well known in the art.

Nucleic acid molecules and fragments thereof of the present invention may also be employed to obtain nucleic acid homologues. Such homologues include the nucleic acid molecule of other organisms (*e.g.*, monkey, cow, cat) including the nucleic acid molecules that encode, in whole or in part, protein homologues of plant species or other organisms, sequences of genetic elements such as promoters and transcriptional regulatory elements. Methods for forming such libraries are well known in the art. Such homologue molecules may differ in their nucleotide sequences from those found SEQ ID: 3 or complement thereof. The nucleic acid molecules of the present invention therefore also include molecules that, although capable of specifically hybridizing with the nucleic acid molecules may lack "complete complementarity."

In one embodiment, the nucleic acid molecules of the present invention include molecules that encode the canine COX-1 protein, its promoter, or flanking gene sequences. In a preferred embodiment, a canine nucleic acid of the present invention will specifically hybridize to a nucleic acid molecule having SEQ ID: 1 or its compliments. In one aspect of the present invention, a canine nucleic acid molecule of the present invention will comprise SEQ ID: 1 or its compliment.

Fragment canine COX-1 nucleic acid molecules may encode significant portion(s) of, or indeed most of, the canine COX-1 protein. Preferably, a fragment canine COX-1 nucleic acid molecule is identical or complementary to at least 20 contiguous nucleotides in SEQ ID: 1. More preferably it comprises at least 25 nucleotides in SEQ ID: 1. Even more preferably it comprises at least 50 nucleotides in SEQ ID: 1. Most preferably, it comprises at least 100 nucleotides in SEQ ID: 1. In a preferred embodiment, a fragment canine COX-1 nucleic acid molecule comprises at least one nucleotide that is not found in a corresponding position in canine COX-1. In another preferred embodiment, the fragment canine COX-1 molecule exhibits canine COX-1 enzymatic activity.

In another embodiment of the present invention, a nucleic acid molecule of the present invention shares between 100% and 90% sequence identity with the canine COX-1 nucleic acid sequence set forth in SEQ ID: 1 or its complement. In a further aspect of the present invention, a nucleic acid molecule of the present invention shares between 100% and 95% sequence identity with the canine nucleic acid sequence set forth in SEQ ID: 1 or its complement. In a more preferred aspect of the present invention, a nucleic acid molecule of the present invention shares between 100% and 98% sequence identity with SEQ ID: 1 or its complement. In a still more preferred aspect of the present invention, a nucleic acid molecule of the present invention shares between 100% and 99% sequence identity with SEQ ID: 1 or its complement.

The present invention also provides a substantially-purified canine COX-2 protein or fragment thereof encoded by a nucleic acid sequence which specifically hybridizes to SEQ ID: 4 or its complement.

The present invention also provides a substantially-purified canine COX-1 protein having SEQ ID: 2.

The present invention also provides a substantially-purified canine COX-2 protein or fragment thereof comprising at least 10 consecutive amino acids of SEQ ID: 4.

The present invention also provides an antibody capable of specifically binding to the substantially-purified canine COX-2 protein.

The present invention also provides a substantially-purified canine COX-2 protein or fragment thereof comprising at least 10 consecutive amino acids of SEQ ID: 4.

Nucleic acid molecules of the present invention may be used to find any canine COX-2 nucleic acid homologue. Nucleic acid molecules of the present invention may also be used to obtain any mammalian COX-2 homologue.

Any of a variety of methods may be used to obtain one or more of the above-described nucleic acid molecules. The following individual references (each of which is individually herein incorporated by reference) each describes useful method of obtaining nucleic acid molecules.

Zamechik et al., Proc. Natl. Acad. Sci. (U.S.A.) 83:4143-4146 (1986).
Goodchild et al., Proc. Natl. Acad. Sci. (U.S.A.) 85:5507-5511 (1988).
Wickstrom et al., Proc. Natl. Acad. Sci. (U.S.A.) 85:1028-1032 (1988).
Holt et al., Molec. Cell. Biol. 8:963-973 (1988).
Gerwirtz et al., Science 242:1303-1306 (1988).
Anfossi et al., Proc. Natl. Acad. Sci. (U.S.A.) 86:3379-3383 (1989).

Becker et al., EMBO J. 8:3685-3691 (1989).

Automated nucleic acid synthesizers may be employed for this purpose. In lieu of such synthesis, the disclosed nucleic acid molecules may be used to define a pair of primers that can be used with the polymerase chain reaction to amplify and obtain any desired nucleic acid molecule or fragment. Each of the following references (each of which is individually herein incorporated by reference) individually describe useful methods to define primers that can be used with the polymerase chain reaction.

Mullis et al., Cold Spring Harbor Symp. Quant. Biol. 51:263-273 (1986).
Erlich et al., European Patent 50,424.
European Patent 84,796.
European Patent 258,017.
European Patent 237,362.
Mullis, European Patent 201,184.
Mullis et al., U.S. Patent 4,683,202.
Erlich, U.S. Patent 4,582,788.
Saiki, R. et al., U.S. Patent 4,683,194.

The canine COX-2 promoter sequence(s) and canine COX-2 flanking sequences can also be obtained using the SEQ ID: 3 sequence provided herein. In one embodiment, such sequences are obtained by incubating oligonucleotide probes of canine COX-2 oligonucleotides with members of genomic canine libraries and recovering clones that hybridize to the probes. In a second embodiment, methods of "chromosome walking," or 3' or 5' RACE may be used to obtain such sequences. Each of the following individual references contains useful descriptions or "chromosome walking," or 3' or 5' RACE.

Frohman, M.A. *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.) 85*:8998-9002 (1988).

Ohara, O. *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.) 86*:5673-5677 (1989).

B. Pr teins and Peptides

A second class of preferred agents comprises the canine COX-1 and COX-2 proteins, and their peptide fragments, fusion proteins, and analogs. Canine COX-1 and COX-2 proteins may be produced via chemical synthesis, or more preferably, by expressing canine COX-1- or COX-2-encoding cDNA in a suitable bacterial or eukaryotic host. Most preferably, the subsequence of such cDNA that encodes canine COX-1 or COX-2 may be used for this purpose (SEQ ID: 1 or 3). Suitable methods for expression are described by Sambrook, J., *et al.*, (*Molecular Cloning, a Laboratory Manual, 2nd Edition, Cold Spring Harbor Press*, Cold Spring Harbor, New York (1989)), or similar texts.

A "canine COX-2 fragment" is a peptide or polypeptide the amino acid sequence of which comprises a subset of the amino acid sequence of canine COX-2 protein. Preferably a canine COX-2 fragment molecule is identical or complementary to at least one region which corresponds to a contiguous 10 amino acid sequence of SEQ ID: 4. More preferably, the canine COX-2 fragment is identical or complementary to at least one region which corresponds to a contiguous 50 amino acids of SEQ ID: 4. Even more preferably, the canine COX-2 fragment is identical or complementary to at least one region which responds to a contiguous 100 amino acids of SEQ ID: 4. A canine COX-2 protein or fragment thereof that comprises one or more additional non-canine COX-2 peptide regions is a "canine COX-2 fusion" protein. Such molecules may be derivatized to contain carbohydrate or other moieties (for example, keyhole limpet hemocyanin). As in the case of canine COX-2 protein, the fragments and fusions of the present invention are preferably produced via recombinant means.

The analogs of the canine COX-2 protein molecules comprise canine COX-2 proteins, fragments or fusions in which non-essential, nor relevant, amino acid residues have been added, replaced, or deleted. In other words, in analogs of canine COX-2 protein, no amino acids have been added, replaced or deleted which w uld

affect the activity of the analog protein relative to parent canine COX-2. An example of such an analog is the COX-2 protein of a non-canine species, such as a primate, a cow, a cat, and the like. Such analogs can readily be obtained by any of a variety of methods. Most preferably, as indicated above, the disclosed SEQ ID: 3 will be used to define a pair of primers that may be used to isolate the canine COX-2-encoding nucleic acid molecules from any desired species. Such molecules can be expressed to yield canine COX-2 analogs by recombinant means.

Accordingly, in one embodiment the present invention provides a substantially-purified canine COX-1 protein or a fragment thereof wherein the canine COX-1 protein or its fragment is encoded by a nucleic acid sequence which specifically hybridizes to SEQ ID: 1 or a complement of SEQ ID: 1.

In another embodiment, the present invention provides a substantially-purified canine COX-1 protein having SEQ ID: 2.

In yet another embodiment, the present invention provides a substantially-purified canine COX-1 protein or a fragment thereof comprising at least 10 consecutive amino acids of SEQ ID: 2. Preferably the substantially-purified canine COX-1 protein or a fragment thereof is a fusion protein.

The present invention further provides a substantially-purified canine COX-2 protein or a fragment thereof encoded by a nucleic acid sequence which specifically hybridizes to SEQ ID: 4 or its complement. Preferably, the substantially-purified canine COX-2 protein or fragment specifically hybridizes to SEQ ID: 4. Alternatively, the substantially-purified canine COX-2 protein or fragment thereof can specifically hybridize to the complement of SEQ ID: 4.

The present invention further provides a substantially-purified canine COX-2 protein having SEQ ID: 4. The present invention also provides a substantially-purified canine COX-2 protein or a fragment thereof wherein the protein or the fragment thereof comprises at least 10 consecutive amino acids of SEQ ID: 4. In one embodiment the canine COX-2 protein or fragment thereof is a fusion protein.

C. Antibodies Reactive Against canine COX-1 or canine COX-2

One aspect of the present invention concerns antibodies, single-chain antigen binding molecules, or other proteins that specifically bind to canine COX-2 protein and its analogs, fusions or fragments. Such antibodies are "anti-canine COX-2 antibodies," and may be used, for example to measure canine COX-1 or canine COX-2 protein. As used herein, an antibody or peptide is the to "specifically bind" to canine COX-1 or canine COX-2 if such binding is not competitively inhibited by the presence of non-canine COX molecules.

Nucleic acid molecules that encode all or part of the canine COX-2 protein can be expressed, via recombinant means, to yield canine COX-2 protein or peptides that can in turn be used to elicit antibodies that are capable of binding canine COX-2. Such antibodies may be used in immunodiagnostic assays. Such canine COX-2-encoding molecules, or their fragments may be a "fusion" molecule (i.e. a part of a larger nucleic acid molecule) such that, upon expression, a fusion protein is produced.

The antibodies that specifically bind canine COX-2 proteins and protein fragments may be polyclonal or monoclonal, and may comprise intact immunoglobulins, of antigen binding portions of immunoglobulins (such as (F(ab'), $F(ab')_2$) fragments, or single-chain immunoglobulins producable, for example, via recombinant means. It is understood that practitioners are familiar with the standard resource materials which describe specific conditions and procedures for the construction, manipulation and isolation of antibodies (*see*, for example, Harlow and Lane, *In Antibodies: A Laboratory Manual,* Cold Spring Harbor Press, Cold Spring Harbor, New York (1988)).

Murine monoclonal antibodies are particularly preferred. BALB/c mice are preferred for this purpose, however, equivalent strains may also be used. The animals are preferably immunized with approximately 25 μg of purified canine COX-2 protein

(r fragment thereof) that has been emulsified with a suitable adjuvant (such as TiterMax adjuvant (Vaxcel, Norcross, GA)). Immunization is preferably conducted at two intramuscular sites, one intraperitoneal site, and one subcutaneous site at the base of the tail. An additional i.v. injection of approximately 25 μg of antigen is preferably given in normal saline three weeks later. After approximately 11 days following the second injection, the mice may be bled and the blood screened for the presence of anti-canine COX-2 antibodies. Preferably, a direct binding ELISA is employed for this purpose.

Most preferably, the mouse having the highest antibody titer is given a third i.v. injection of approximately 25 μg of canine COX-2 protein, fragment, or fusion. The splenic leukocytes from this animal may be recovered 3 days later, and are then permitted to fuse, most preferably, using polyethylene glycol, with cells of a suitable myeloma cell line (such as, for example, the P3X63Ag8.653 myeloma cell line). Hybridoma cells are selected by culturing the cells under "HAT" (hypoxanthine-aminopterin-thymine) selection for about one week. The resulting clones may then be screened for their capacity to produce monoclonal antibodies ("mAbs) to canine COX-2 protein, preferably by direct ELISA.

In one embodiment, anti-canine COX-2 monoclonal antibodies are isolated using canine COX-2 fusions, or conjugates, as immunogens. Thus, for example, a group of mice can be immunized using a canine COX-2 fusion protein emulsified in Freund's complete adjuvant (approximately 50 μg of antigen per immunization). At three week intervals, an identical amount of antigen is emulsified in Freund's incomplete adjuvant and used to immunize the animals. Ten days following the third immunization, serum samples are taken and evaluated for the presence of antibody. If antibody titers are too low, a fourth booster can be employed. Polysera capable of binding canine COX-2 at 1:5,000 dilution can also be obtained using this method.

In a preferred procedure for obtaining monoclonal antibodies, the spleens of the above-described immunized mice are removed, disrupted, and immune

splenocytes are isolated over a ficoll gradient. The isolated splenocytes are fused, using polyethylene glycol with BALB/c-derived HGPRT (hypoxanthine guanine phosphoribosyl transferase) deficient P3x63xAg8.653 plasmacytoma cells. The fused cells are plated into 96 well microtiter plates and screened for hybridoma fusion cells by their capacity to grow in culture medium supplemented with hypothanthine, aminopterin and thymidine for approximately 2-3 weeks. On average, out of every 10^6 spleen cells subjected to fusion yields a viable hybridoma. A typical spleen yields 5-10 x 10^7 spleen cells.

Hybridoma cells that arise from such incubation are preferably screened for their capacity to produce an immunoglobulin that binds to canine COX-2 protein. An indirect ELISA may be used for this purpose. In brief, the supernatants of hybridomas are incubated in microtiter wells that contain immobilized canine COX-2 protein. After washing, the titer of bound immunoglobulin can be determined using, for example, a goat anti-mouse antibody conjugated to horseradish peroxidase. After additional washing, the amount of immobilized enzyme is determined (for example, through the use of a chromogenic substrate). Such screening is performed as quickly as possible after the identification of the hybridoma in order to ensure that a desired clone is not overgrown by non-secreting neighbors. Preferably, the fusion plates are screened several times since the rates of hybridoma growth vary. In a preferred embodiment, a different antigenic form of canine COX-2 may be used to screen the hybridoma. Thus, for example, the splenocytes may be immunized with one canine COX-2 immunogen, but the resulting hybridomas can be screened using a different canine COX-2 immunogen.

As discussed below, such antibody molecules or their fragments may be used for diagnostic purposes. Where the antibodies are intended for diagnostic purposes, it may be desirable to derivatize them, for example with a ligand group (such as biotin) or a detectable marker group (such as fluorescent group, a radioisotope or an enzyme).

The ability to produce antibodies that bind canine COX-2 molecules permits the identification of mimetic compounds of canine COX-2. A "mimetic compound" of canine COX-2 is a compound that is not canine COX-2, or a fragment of canine COX-2, but which nonetheless exhibits an ability to specifically bind to anti-canine COX-2 antibodies. Such molecules can be used to elicit anti-canine COX-2 antibodies, and thus, may be used to assist diagnosis of canine COX-2 related disorders.

Accordingly, in one embodiment the present invention provides an antibody capable of specifically binding to a substantially-purified canine COX-1 protein having SEQ ID: 2; preferably the antibody is detectably labeled.

In another embodiment, the present invention provides an antibody capable of specifically binding to the substantially-purified canine COX-2 protein having SEQ ID: 4. Preferably the antibody is detectably labeled.

III. Uses of the Molecules of the Invention

In another embodiment the present invention provides plasmid DNA vectors for use in the expression of the canine COX-1 or COX-2 proteins. These vectors contain the DNA sequences described above which code for the polypeptides of the invention. Appropriate vectors which can transform eukaroytic cells, including mammalian cells and microorganisms capable of expressing the canine COX-1 or COX-2 proteins include expression vectors comprising nucleotide sequences coding for the canine COX-1 or COX-2 proteins joined to transcriptional and translational regulatory sequences which are selected according to the host cells used.

Vectors incorporating modified sequences as described above are included in the present invention and are useful in the production of the canine COX-1 or COX-2 polypeptides. The vector employed in the method also contains selected regulatory sequences in operative association with the DNA coding sequences of the invention

which are capable of directing the replication and expression thereof in selected host cells.

Transfer of a nucleic acid that encodes for a protein can result in expression or overexpression of that protein in a transformed cell. One or more of the proteins or fragments thereof encoded by nucleic acid molecules of the present invention may be expressed or overexpressed in a transformed cell. Particularly, any of the canine COX-1 or COX-2 proteins or fragments thereof may be expressed or overexpressed in a transformed cell. Such expression or overexpression may be the result of transient or stable transfer of the exogenous genetic material. "Exogenous genetic material" is any genetic material, whether naturally occurring or otherwise, from any source, which genetic material is capable of being inserted into an organism.

A construct or vector may include a promoter to express the protein or protein fragment of choice. Preferably, the promoter of the present invention is a COX-specific promoter. Promoters that can be used in the present invention include:

glucose-6 phosphotase promoter	Yoshiuchi *et al.*, *J. Clin. Endocrin. Metab. 83*:1016-1019 (1998)
interleukin-1 alpha promoter	Mori and Prager, *Leuk. Lymphoma 26*:421-433 (1997)
CMV promoter	Tong *et al.*, *Anticancer Res. 18*:719-725 (1998); Norman *et al.*, *Vaccine 15*:801-803 (1997)
RSV promoter	Elshami *et al.*, *Cancer Gene Ther. 4*:213-221 (1997); Baldwin *et al.*, *Gene Ther. 4*:1142-1149 (1997)
SV40 promoter	Harms and Splitter, *Hum. Gene Ther. 6*:1291-1297 (1995)

CD11c integrin gene promoter	Corbi and Lopez- Rodriguez, *Leuk. Lymphoma 25*:415-425 (1997)
GM-CSF promoter	Shannon *et al.*, *Crit. Rev. Immunol. 17*:301-323 (1997)
interleukin-5R alpha promoter	Sun *et al.*, *Curr. Top. Microbiol. Immunol 211*:173-187 (1996)
interleukin-2 promoter	Serfing *et al.*, *Biochim. Biophys. Acta 1263*:181-200 (1995) O'Neill *et al.*, *Transplant Proc. 23*:2862-2866 (1991)
c- fos promoter	Janknecht, *Immunobiology 193*:137-142 (1995) Janknecht *et al.*, *Carcinogenesis 16*:443-450 (1995) Takai *et al.*, *Princess Takamatsu Symp. 22*:197-204 (1991)
h-ras promoter	Rachal *et al.*, *EXS 64*:330-342 (1993)
DMD gene promoter	Ray *et al.*, *Adv. Exp. Med. Biol. 280*:107-111 (1990)

Promoters suitable for expression of the canine COX-1 or COX-2 proteins or fragments of either of the present invention in bacteria have been described by Hawley and McClure, *Nucleic Acids Res. 11*:2237-2255 (1983), Harley and Reynolds, *Nucleic Acids Res. 15*:2343-2361 (1987) have also described promoters suitable for

expression of the canine COX-1 or COX-2 proteins or fragments of either of the present invention in bacteria. Such promoters include, for example:

the *rec*A promoter-	Fernandez de Henestrosa *et al.*, *FEMS Microbiol. Lett. 147*:209-213 (1997) Nussbaumer *et al.*, *FEMS Microbiol. Lett. 118*:57-63 (1994) Weisemann *et al.*, *Biochimie 73*: 457-470 (1991)),
the Ptac promoter	Hasan *et al.*, *Gene 56*:141-151 (1987); Marsh, *Nucleic Acids Res. 14*:3603 (1986)
Ptac-*rec*A hybrid promoter	

It is preferred that the particular promoter selected is capable of causing sufficient expression to result in the production of an effective amount of the canine COX-1 or COX-2 proteins or fragments of either to cause the desired phenotype.

Constructs or vectors may also include with the coding region of interest a nucleic acid sequence that acts, in whole or in part, to terminate transcription of that region.

Translational enhancers may also be incorporated as part of the vector DNA. DNA constructs could contain one or more 5' non-translated leader sequences that may serve to enhance expression of the gene products from the resulting mRNA transcripts. Such sequences may be derived from the promoter selected to express the gene or can be specifically modified to increase translation of the mRNA. Such regions may also be obtained from viral RNAs, from suitable eukaryotic genes, or from a synthetic gene sequence.

A vector or construct may also include a screenable marker. Screenable markers may be used to monitor expression. Exemplary screenable markers include:

β-glucuronidase encoded by the *uid*A gene (GUS)	Jefferson, *Plant Mol. Biol. Rep. 5:* 387-405 (1987) Jefferson *et al., EMBO J. 6:* 3901-3907 (1987)
β-lactamase	Sutcliffe *et al., Proc. Natl. Acad. Sci. (U.S.A.) 75*: 3737-3741 (1978)
luciferase	Clontech, Palo Alto, CA, USA Ow *et al., Science 234*: 856-859 (1986)
β-galactosidase	Clontech, Palo Alto, CA, USA
GST	Stratagene)
Protein A	Calbiochem
blue fluorescent protein	Clontech, Palo Alto, CA, USA
fluorescent green protein	Clontech, Palo Alto, CA, USA

Included within the terms "selectable or screenable marker genes" are also genes which encode a secretable marker whose secretion can be detected as a means of identifying or selecting for transformed cells. Examples include markers which encode a secretable antigen that can be identified by antibody interaction, or even secretable enzymes which can be detected by catalytic reactions. Secretable proteins fall into a number of classes, including small, diffusible proteins which are detectable, (*e.g.*, by ELISA), small active enzymes which are detectable in extracellular solution (*e.g.*, α-amylase, β-lactamase, phosphinothricin transferase), or proteins which are inserted or trapped in the cell membrane (such as proteins which include a leader

sequence). Other possible selectable and/or screenable marker genes are apparent to those of skill in the art.

As another aspect of the present invention, there is provided a method for producing the canine COX-1 or COX-2 proteins. Suitable cells or cell lines may be mammalian, insect or yeast cells. For example, the canine COX-1 or COX-2 proteins or fragments of either of the present invention is expressed in a yeast cell, preferably *Saccharomyces cerevisiae*. The canine COX-1 or COX-2 proteins or fragments of either of the present invention can be expressed in *S. cerevisiae* by fusing it to the N-terminus of the URA3, CYC1 or ARG3 genes (Guarente and Ptashne, *Proc. Natl. Acad. Sci. (U.S.A.) 78*:2199-2203 (1981); Rose *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.) 78*:2460-2464 (1981); and Crabeel *et al.*, *EMBO J. 2*:205-212 (1983)). Alternatively, the canine COX-1 or COX-2 proteins or fragments of either of the present invention can be fused to either the PGK or TRP1 genes (Tuite *et al.*, *EMBO J. 1*:603-608 (1982); and Dobson *et al.*, *Nucleic Acids. Res. 11*:2287-2302 (1983)). More preferably, the canine COX-1 or COX-2 proteins or fragments of either of the present invention are expressed as a mature protein (Hitzeman *et al.*, *Nature 293*:717-722 (1981); Valenzuela *et al.*, *Nature 298*:347-350 (1982); and Derynck *et al.*, *Nucleic Acids Res. 11*:1819-1837 (1983)).

Native and engineered yeast promoters suitable for use in the present invention have been reviewed by Romanos *et al.*, *Yeast 8*:423-488 (1992) Most preferably, the canine COX-1 or COX-2 proteins or fragments of either of the present invention is secreted by the yeast cell (Blobel and Dobberstein, *J. Cell Biol. 67*:835-851 (1975); Kurjan and Herskowitz, *Cell 30*:933-943 (1982); Bostian *et al.*, *Cell 36*:741-751 (1984); Rothman and Orci, *Nature 355*:409-415 (1992); Julius *et al.*, *Cell 32*:839-852 (1983); and Julius *et al.*, *Cell 36*:309-318 (1984)).

Where desired, insect cells may be utilized as host cells in the method of the present invention. See, e.g., V. A. Luckow, *Protein Eng.* J. L. Cleland., Wiley-Liss, New York, NY: 183-2180 (1996) and references cited therein. In addition, general

methods for expression of foreign genes in insect cells using baculovirus vectors are described in: O'Reilly *et al.*, *Baculovirus Expression Vectors: A Laboratory Manual.* New York, W.H. Freeman and Company (1992), and King and Possee, *The Baculovirus Expression System: A Laboratory Guide*, London, Chapman & Hall. An expression vector is constructed comprising a baculovirus transfer vector, in which a strong baculovirus promoter (such as the polyhedrin promoter) drives transcription of a eukaryotic secretion signal peptide coding region, which is translationally joined to the coding region for the multi-functional protein. For example, the plasmid pVL1393 (Invitrogen Corp., San Diego, California, U.S.A.) can be used. After construction of the vector carrying the gene encoding the multi-functional protein, two micrograms of this DNA is co-transfected with one microgram of baculovirus DNA into *Spodoptera frugiperda* insect cells, strain Sf9. Alternatively, recombinant baculoviruses can be created using a baculovirus shuttle vector system (Luckow *et al.*, *J. Virol. 67*: 4566-4579 (1993)), now marketed as the Bac-To-Bac™ Expression System (Life Technologies, Inc. Rockville, MD). Pure recombinant baculovirus carrying the multi-functional protein is used to infect cells cultured, for example, in Excell 401 serum-free medium (JRH Biosciences, Lenexa, Kansas) or Sf900-II (Life Technologies, Inc.). The multi-functional protein secreted into the medium can be recovered by standard biochemical approaches. Supernatants from mammalian or insect cells expressing the multi-functional proteins can be first concentrated using any of a number of commercial concentration units.

Most preferably, mammalian cells can be used to express the nucleic acid molecules of the present invention. Preferably, the nucleic acid molecules of the present invention are cloned into a suitable retroviral vector (*see, e.g.*. Dunbar *et al.*, *Blood 85*:3048-3057 (1995); Baum *et al.*, *J. Hematother. 5*: 323-329 (1996); Bregni *et al.*, *Blood 80*:1418-1422 (1992); Boris-Lawrie and Temin, *Curr. Opin. Genet. Dev. 3*:102-109 (1993); Boris-Lawrie and Temin, *Annal. New York Acad. Sci. 716*:59-71 (1994); Miller, *Current Top. Microbiol. Immunol. 158*:1-24 (1992)), adenovirus

vector (Berkner, *BioTechniques 6*:616-629 (1988); Berkner, *Current Top. Microbiol. Immunol. 158*:39-66 (1992); Brody and Crystal, *Annal. New York Acad. Sci. 716*:90-103 (1994); Baldwin *et al.*, *Gene Ther. 4*:1142-1149 (1997)), RSV, MuSV, SSV, MuLV (Baum *et al.*, *J. Hematother. 5*: 323-329 (1996)), AAV (Chen *et al.*, *Gene Ther. 5*:50-58 (1998); Hallek *et al.*, *Cytokines Mol. Ther. 2*: 69-79 (1996)), AEV, AMV, or CMV (Griffiths *et al.*, *Biochem. J. 241*: 313-324 (1987)).

General methods and compositions for transforming a eukaryotic cell, bacteria and other microorganisms are known in the art (*see*, for example, Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., (1989)).

Technology for introduction of DNA into cells is well known to those of skill in the art. Four general methods for delivering a gene into cells have been described: (1) chemical methods (Graham and van der Eb, *Virology 54*:536-539 (1973)); (2) physical methods such as microinjection (Capecchi, *Cell* 22:479-488 (1980)), electroporation (Wong and Neumann, *Biochem. Biophys. Res. Commun. 107*:584-587 (1982); Fromm *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.) 82*:5824-5828 (1985); U.S. Patent No. 5,384,253); and the gene gun (Johnston and Tang, *Methods Cell Biol. 43*:353-365 (1994)); (3) viral vectors (Clapp, *Clin. Perinatol. 20*:155-168 (1993); Lu *et al.*, *J. Exp. Med. 178*:2089-2096 (1993); Eglitis and Anderson, *Biotechniques, 6*:608-614 (1988)); and (4) receptor-mediated mechanisms (Curiel *et al.*, *Hum. Gen. Ther. 3*:147-154 (1992), Wagner *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.) 89*:6099-6103 (1992)).

Transformation can be achieved using methods based on calcium phosphate precipitation, polyethylene glycol treatment, electroporation, and combinations of these treatments (see for example Potrykus *et al.*, *Mol. Gen. Genet. 205*:193-200 (1986); Lorz *et al.*, *Mol. Gen. Genet. 199*:178 (1985); Fromm *et al.*, *Nature 319*:791 (1986); Uchimiya *et al.*, *Mol. Gen. Genet. 204*:204 (1986); Marcotte *et al.*, *Nature 335*:454-457 (1988)).

Assays for gene expression based on the transient expression of cloned nucleic acid constructs have been developed by introducing the nucleic acid molecules into cells by polyethylene glycol treatment, electroporation, or particle bombardment (Marcotte *et al., Nature 335*: 454-457 (1988); McCarty *et al., Cell 66:* 895-905 (1991); Hattori *et al., Genes Dev. 6:* 609-618 (1992); Goff *et al., EMBO J. 9:* 2517-2522 (1990)). Transient expression systems may be used to functionally dissect gene constructs.

In one embodiment, the canine COX-2 molecules of the present invention are used to determine whether an individual has a mutation affecting the level (i.e., the concentration of canine COX-2 mRNA or protein in a sample, etc.) or pattern (i.e., the kinetics of expression, rate of decomposition, stability profile, etc.) of the canine COX-2 expression (collectively, the "canine COX-2 Response" of a cell or bodily fluid) (for example, a mutation in the canine COX-2 gene, or in a regulatory region(s) or other gene(s) that control or affect the expression of canine COX-2), and being predictive of dogs who would be predisposed to, for example inflammatory joint disease, and other disorders. Such COX-2 related disorders include, but are not limited to, arthritis, rheumatoid arthritis, spondyloarthropathies, gouty arthritis, osteoarthritis, systemic lupus erythematosus and juvenile arthritis, auto-immune disease, allograft rejection, asthma, bronchitis, tendinitis, bursitis, and skin-related conditions such as psoriasis, eczema, burns, and dermatitis, post-operative inflammation including from ophthalmic surgery such as cataract surgery and refractive surgery, gastrointestinal conditions such as inflammatory bowel syndrome, and ulcerative colitis, neoplasia, such as colorectal cancer, and cancer of the breast, lung, prostate, bladder, cervix, and skin, vascular diseases, migraine headaches, periateritis nodosa, thyroiditis, aplastic anemia, Hodgkin's disease, sclerodoma, rheumatic fever, type I diabetes, neuromuscular junction disease including multiple sclerosis, sarcoidosis, nephrotic syndrome, Behcet's syndrome, polymyositis, gingivitis, nephritis, hypersensitivity, conjunctivitis, swelling occurring after injury,

myocardial ischemia, myochardial infarction, ophthalmic diseases, such as retinitis, retinopathies, uveitis, ocular photophobia, and of acute injury to the eye tissue, allergic rhinitis, respiratory distress syndrome, endotoxin shock syndrome, atherosclerosis, pulmonary inflammation such as from viral and bacterial infections and from cystic fibrosis, central nervous system disorders, such as cortical dementias including Alzheimer's disease, and central nervous system damage resulting from stroke, ischemia, and trauma.

As used herein, the canine COX-2 Response manifested by a cell or bodily fluid is the to be "altered" if it differs from the canine COX-2 Response of cells or of bodily fluids of normal individuals. Such alteration may be manifested by either abnormally increased or abnormally diminished canine COX-2 Response. To determine whether a canine COX-2 Response is altered, the canine COX-2 Response manifested by the cell or bodily fluid of the canine is compared with that of a similar cell sample (or bodily fluid sample) of normal individuals. As will be appreciated, it is not necessary to re-determine the canine COX-2 Response of the cell sample (or bodily fluid sample) of normal dogs each time such a comparison is made; rather, the canine COX-2 Response of a particular canines may be compared with previously obtained values of normal dogs.

In one sub-embodiment, such an analysis is conducted by determining the presence and/or identity of polymorphism(s) in the canine COX-2 gene or its flanking regions which are associated with a disorder.

Any of a variety of molecules can be used to identify such polymorphism(s). In one embodiment, the canine COX-2 cDNA sequence (or a sub-sequence thereof) may be employed as a marker nucleic acid molecule to identify such polymorphism(s). Alternatively, such polymorphisms can be detected through the use of a marker nucleic acid molecule or a marker protein that is genetically linked to (i.e., a polynucleotide that co-segregates with) such polymorphism(s). In a preferred aspect of this embodiment, such marker nucleic acid molecules will have the nucleotide

sequence of a polynucleotide that is closely genetically linked to such polymorphism(s). Polynucleotide markers that map to such locations are well known and can be employed to identify such polymorphism(s).

In an alternative embodiment, such polymorphisms can be detected through the use of a marker nucleic acid molecule that is physically linked to such polymorphism(s). For this purpose, marker nucleic acid molecules comprising a nucleotide sequence of a polynucleotide located within 1 mb of the polymorphism(s), and more preferably within 100 kb of the polymorphism(s), and most preferably within 10 kb of the polymorphism(s) can be employed.

The genomes of animals and plants naturally undergo spontaneous mutation in the course of their continuing evolution (Gusella, *Ann. Rev. Biochem.* 55:831-854 (1986)).

A "polymorphism" in the canine COX-2 gene or its flanking regions is a variation or difference in the sequence of the canine COX-2 gene or its flanking regions that arises in some of the members of a species. The variant sequence and the "original" sequence co-exist in the species' population. In some instances, such co-existence is in stable or quasi-stable equilibrium.

A polymorphism is thus the to be "allelic," in that, due to the existence of the polymorphism, some members of a species may have the original sequence (i.e. the original "allele") whereas other members may have the variant sequence (i.e. the variant "allele"). In the simplest case, only one variant sequence may exist, and the polymorphism is thus the to be di-allelic. In other cases, the species' population may contain multiple alleles, and the polymorphism is termed tri-allelic, etc. A single gene may have multiple different unrelated polymorphisms. For example, it may have a di-allelic polymorphism at one site, and a multi-allelic polymorphism at another site.

The variation that defines the polymorphism may range from a single nucleotide variation to the insertion or deletion of extended regions within a gene. In some cases, the DNA sequence variations are in regions of the genome that are

characterized by short tandem repeats (STRs) that include tandem di- or tri-nucleotide repeated motifs of nucleotides. Polymorphisms characterized by such tandem repeats are referred to as "variable number tandem repeat" ("VNTR") polymorphisms. VNTRs have been used in identity and paternity analysis (Weber, U.S. Patent 5,075,217; Armour, *et al.*, *FEBS Lett. 307*:113-115 (1992); Jones, *et al.*, *Eur. J. Haematol. 39*:144-147 (1987); Horn, *et al.*, PCT Application WO91/14003; Jeffreys, European Patent Application 370,719; Jeffreys, U.S. Patent 5,175,082); Jeffreys. *et al.*, *Amer. J. Hum. Genet. 39*:11-24 (1986); Jeffreys. *et al.*, *Nature 316*:76-79 (1985); Gray, *et al.*, *Proc. R. Acad. Soc. Lond. 243*:241-253 (1991); Moore, *et al.*, *Genomics 10*:654-660 (1991); Jeffreys, *et al.*, *Anim. Genet. 18*:1-15 (1987); Hillel, *et al.*, *Anim. Genet. 20*:145-155 (1989); Hillel, *et al.*, *Genet. 124*:783-789 (1990)).

The detection of polymorphic sites in a sample of DNA may be facilitated through the use of nucleic acid amplification methods. Such methods specifically increase the concentration of polynucleotides that span the polymorphic site, or include that site and sequences located either distally or proximally to it. Such amplified molecules can be readily detected by gel electrophoresis or other means.

The most preferred method of achieving such amplification employs the polymerase chain reaction ("PCR") (Mullis, *et al.*, *Cold Spring Harbor Symp. Quant. Biol. 51*:263-273 (1986); Erlich *et al.*, European Patent Appln. 50,424; European Patent Appln. 84,796, European Patent Application 258,017, European Patent Appln. 237,362; Mullis, European Patent Appln. 201,184; Mullis *et al.*, U.S. Patent No. 4,683,202; Erlich, U.S. Patent No. 4,582,788; and Saiki, *et al.*, U.S. Patent No. 4,683,194), using primer pairs that are capable of hybridizing to the proximal sequences that define a polymorphism in its double-stranded form.

In lieu of PCR, alternative methods, such as the "Ligase Chain Reaction" ("LCR") may be used (Barany, *Proc. Natl. Acad. Sci. (U.S.A.) 88*:189-193 (1991). LCR uses two pairs of oligonucleotide probes to exponentially amplify a specific target. The sequences of each pair of oligonucleotides is selected to permit the pair to

hybridize to abutting sequences of the same strand of the target. Such hybridization forms a substrate for a template-dependent ligase. As with PCR, the resulting products thus serve as a template in subsequent cycles and an exponential amplification of the desired sequence is obtained.

LCR can be performed with oligonucleotides having the proximal and distal sequences of the same strand of a polymorphic site. In one embodiment, either oligonucleotide will be designed to include the actual polymorphic site of the polymorphism. In such an embodiment, the reaction conditions are selected such that the oligonucleotides can be ligated together only if the target molecule either contains or lacks the specific nucleotide that is complementary to the polymorphic site present on the oligonucleotide. Alternatively, the oligonucleotides may be selected such that they do not include the polymorphic site (see, Segev, PCT Application WO 90/01069).

The "Oligonucleotide Ligation Assay" ("OLA") may alternatively be employed (Landegren, *et al., Science 241:*1077-1080 (1988)). The OLA protocol uses two oligonucleotides which are designed to be capable of hybridizing to abutting sequences of a single strand of a target. OLA, like LCR, is particularly suited for the detection of point mutations. Unlike LCR, however, OLA results in "linear" rather than exponential amplification of the target sequence.

Nickerson, *et al.* have described a nucleic acid detection assay that combines attributes of PCR and OLA (Nickerson, *et al., Proc. Natl. Acad. Sci. (U.S.A.) 87:*8923-8927 (1990)). In this method, PCR is used to achieve the exponential amplification of target DNA, which is then detected using OLA. In addition to requiring multiple, and separate, processing steps, one problem associated with such combinations is that they inherit all of the problems associated with PCR and OLA.

Schemes based on ligation of two (or more) oligonucleotides in the presence of nucleic acid having the sequence of the resulting "di-oligonucleotide", thereby

amplifying the di-oligonucleotide, are also known (Wu, *et al.*, *Genomics 4*:560 (1989)), and may be readily adapted to the purposes of the present invention.

Other known nucleic acid amplification procedures, such as allele-specific oligomers, branched DNA technology, transcription-based amplification systems, or isothermal amplification methods may also be used to amplify and analyze such polymorphisms (Malek, *et al.*, U.S. Patent 5,130,238; Davey, *et al.*, European Patent Application 329,822; Schuster *et al.*, U.S. Patent 5,169,766; Miller, *et al.*, PCT appln. WO 89/06700; Kwoh, *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.) 86*:1173 (1989); Gingeras, *et al.*, PCT application WO 88/10315; Walker, *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.) 89*:392-396 (1992)). All the foregoing nucleic acid amplification methods could be used.

The identification of a polymorphism in the canine COX-2 gene can be determined in a variety of ways. By correlating the presence or absence of inflammatory joint disease in an dog with the presence or absence of a polymorphism in the canine COX-2 gene or its flanking regions, it is possible to diagnose the predisposition of an asymptomatic dog to inflammatory joint disease or other diseases. If a polymorphism creates or destroys a restriction endonuclease cleavage site, or if it results in the loss or insertion of DNA (e.g., a VNTR polymorphism), it will alter the size or profile of the DNA fragments that are generated by digestion with that restriction endonuclease. As such, dogs that possess a variant sequence can be distinguished from those having the original sequence by restriction fragment analysis. Polymorphisms that can be identified in this manner are termed "restriction fragment length polymorphisms" ("RFLPs"). RFLPs have been widely used in human and animal genetic analyses (Glassberg, UK Patent Application 2135774; Skolnick, M.H. *et al.*, *Cytogen. Cell Genet. 32*:58-67 (1982); Botstein, *et al.*, *Ann. J. Hum. Genet. 32*:314-331 (1980); Fischer, *et al.* (PCT Application WO90/13668); Uhlen, PCT Application WO90/11369).

In accordance with this embodiment of the invention, a sample DNA is obtained from a dog's cells. In a preferred embodiment, the DNA sample is obtained from the dog's blood. However, any source of DNA may be used. The DNA is subjected to restriction endonuclease digestion. Canine COX-2 is used as a probe in accordance with the above-described RFLP methods.

The polymorphism obtained in this approach can then be cloned to identify the mutation at the coding region which alters the protein's structure, or at the regulatory region of the gene which affects its expression level. Changes involving promoter interactions with other regulatory proteins can be identified by, for example, gel shift.

Several different classes of polymorphisms may be identified through such methods. Examples of such classes include: (1) polymorphisms present in the canine COX-2 cDNA of different dogs; (2) polymorphisms in non-translated canine COX-2 gene sequences, including the promoter or other regulatory regions of the canine COX-2 gene; (3) polymorphisms in genes whose products interact with canine COX-2 regulatory sequences; (4) polymorphisms in gene sequences whose products interact with the canine COX-2 protein, or to which the canine COX-2 protein binds.

In an alternate sub-embodiment, the evaluation is conducted using oligonucleotide "probes" whose sequence is complementary to that of a portion of canine COX-2 mRNA. Such molecules are then incubated with cell extracts of a patient under conditions sufficient to permit nucleic acid hybridization. For this sub-embodiment, cells of the trabecular meshworks are preferred. The detection of double-stranded probe-mRNA hybrid molecules is indicative of the presence of canine COX-2 mRNA; the amount of such hybrid formed is proportional to the amount of canine COX-2 mRNA. Thus, such probes may be used to ascertain the level and extent of canine COX-2 mRNA production in a patient's cells. Such nucleic acid hybridization may be conducted under quantitative conditions (thereby providing a numerical value of the amount of canine COX-2 mRNA present). Alternatively, the

assay may be conducted as a qualitative assay that indicates either that canine COX-2 mRNA is present, or that its level exceeds a user set, predefined value.

In a second embodiment, the previously described "anti-canine COX-2 antibodies" are employed in an immunodiagnostic assay.

In one sub-embodiment of this aspect of the present invention, one can ascertain the canine COX-2 Response in a biopsy (or a macrophage or other blood cell sample), or other cell sample, or more preferably, in a sample of bodily fluid (especially, blood, serum, plasma, tears, etc.).

The anti-canine COX-2 antibodies of the present invention may thus be used in an immunoassay to assess the presence of canine COX-2. Any of a wide array of immunoassays formats may be used for this purpose (Fackrell, *Clin. Immunoassay 8*:213-219 (1985)), Yolken, *Rev. Infect. Dis. 4*:35 (1982); Collins, In: *Alternative Immunoassays*, John Wiley & Sons, NY (1985); Ngo, *et al.*, In: *Enzyme Mediated Immunoassay*, Plenum Press, NY (1985)).

The simplest immunoassay involves merely incubating an antibody that is capable of binding to a predetermined target molecule with a sample suspected to contain the target molecule. The presence of the target molecule is determined by the presence, and proportional to the concentration, of any antibody bound to the target molecule. In order to facilitate the separation of target-bound antibody from the unbound antibody initially present, a solid phase is typically employed. Thus, for example the sample can be passively bound to a solid support, and, after incubation with the antibody, the support can be washed to remove any unbound antibody.

In more sophisticated immunoassays, the concentration of the target molecule is determined by binding the antibody to a support, and then permitting the support to be in contact with a sample suspected of containing the target molecule. Target molecules that have become bound to the immobilized antibody can be detected in any of a variety of ways. For example, the support can be incubated in the presence of a labeled, second antibody that is capable of binding to a second epitope of the target

molecule. Immobilization of the labeled antibody on the support thus requires the presence of the target, and is proportional to the concentration of the target in the sample. In an alternative assay, the target is incubated with the sample and with a known amount of labeled target. The presence of target molecule in the sample competes with the labeled target molecules for antibody binding sites. Thus, the amount of labeled target molecules that are able to bind the antibody is inversely proportional to the concentration of target molecule in the sample.

In general, immunoassay formats employ either radioactive labels ("RIAs") or enzyme labels ("ELISAs"). RIAs have the advantages of simplicity, sensitivity, and ease of use. Radioactive labels are of relatively small atomic dimension, and do not normally affect reaction kinetics. Such assays suffer, however, from the disadvantages that, due to radioisotopic decay, the reagents have a short shelf-life, require special handling and disposal, and entail the use of complex and expensive analytical equipment. RIAs are described in *Laboratory Techniques and Biochemistry in Molecular Biology*, by Work, *et al.*, North Holland Publishing Company, NY (1978), with particular reference to the chapter entitled "An Introduction to Radioimmune Assay and Related Techniques" by Chard. ELISAs have the advantage that they can be conducted using inexpensive equipment, and with a myriad of different enzymes, such that a large number of detection strategies -- colorimetric, pH, gas evolution, etc. -- can be used to quantitate the assay. In addition, the enzyme reagents have relatively long shelf-lives, and lack the risk of radiation contamination that attends to RIA use. ELISAs are described in *ELISA and Other Solid Phase Immunoassays* (Kemeny, *et al.*, Eds.), John Wiley & Sons, NY (1988).

Anti-canine COX-2 antibodies or canine COX-2 binding molecules may be administered to a dog, and their capacity to bind to canine COX-2 *in vivo* may be determined by ocular examination. Significantly, since such a diagnostic test is relatively rapid, immune responses that require significant time, such as the potential

eliciting of anti-[anti-canine COX-2] antibodies, or the complexing of such antibodies with anti-canine COX-2 antibodies, is not important. In a preferred embodiment, the antibody will be fluorescently labeled, and will be provided to a dog by injection into the dog's circulatory system.

In another aspect of the present invention, a canine COX-2 protein or fragment thereof can be used in assays for screening test substances for the ability to modulate or maintain canine COX-2 activity. In a sub-embodiment, the test substance is an agonist, antagonist, or small molecule inhibitor of the canine COX-2 protein. In another sub-embodiment, the test substance may bind to canine COX-2 substrate. The test substance may also be an agonist, antagonist, or small molecule inhibitor of COX-1.

Assays for screening canine COX-2 activity include, for example, a colorimetric assay (Kaufman et al., *Enzyme* 12:537 (1971), Richards and Greengard, *Biochimica et Biophysica Acta* 304:842-850 (1973), McKnight *et al., J. Biochem.* 267:25208-25212 (1992)) and a HPLC-based separation technique (Buse *et al. Am J. Physiol.* 272: E1080-1088 (1997), Nelson *et al., Am. J. Physiol.* 272: E848-855 (1997)).

Accordingly, the present invention provides a transformed cell having a nucleic acid molecule which comprises an exogenous promoter region linked to a structural nucleic acid sequence, and a 3'non-translated sequence linked to the structural nucleic acid sequence, wherein the exogenous promoter region causes the production of a mRNA molecule; the structural nucleic acid sequence encodes a canine COX-1 gene or a fragment thereof; and the 3' non-translated sequence causes termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of the mRNA molecule. In one embodiment, the structural nucleic acid sequence is in the sense orientation. In an alternative embodiment, the structural nucleic acid sequence is in the antisense orientation.

In another embodiment the present invention provides a transformed cell having a nucleic acid molecule which comprises an exogenous promoter region linked to a structural nucleic acid sequence, and a a 3' non-translated sequence, wherein the exogenous promoter region causes the production of a mRNA molecule; the structural nucleic acid molecule encodes a canine COX-2 gene or a fragment thereof; and the 3' non-translated sequence causes termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of the mRNA molecule. In one embodiment, the structural nucleic acid sequence is in the sense orientation. In an alternative embodiment, the structural nucleic acid sequence is in the antisense orientation.

In another embodiment the present invention provides a method for assaying a canine COX-2 protein in a cell comprising (A) incubating a marker nucleic acid molecule with a complementary nucleic acid molecule obtained from the cell, wherein the incubation is performed under conditions permitting nucleic acid hybridization, and the marker nucleic acid molecule is capable of specifically hybridizing under high stringency conditions to a canine COX-2-encoding nucleic acid molecule or a complement thereof; (B) hybridizing the marker nucleic acid molecule with the complementary nucleic acid molecule; and (C) assaying the complementary nucleic acid, wherein the assay is predictive of the level or pattern of the canine COX-2 protein. In one embodiment, the assay is predictive of the canine COX-2 protein concentration. In another embodiment, the assay is predictive of the canine COX-2 protein pattern. The assay may be performed, for example, by *in situ* hybridization.

In another embodiment the present invention provides a method for diagnosis or prognosis of a COX-2 related condition in a dog which comprises assaying the concentration of a molecule, the concentration being dependent upon the expression of a canine COX-2 gene, and the molecule being assayable in a biological sample from the dog. The biological sample can be practically any biological sample from the animal, for example, skeletal tissue, blood, lymph, or serum. The assayed molecule can, for example, be a protein molecule expressed by the canine COX-2 gene. Alternatively, the assayed molecule can be a nucleic acid molecule such as a mRNA molecule encoded by the canine COX-2 gene, or a cDNA molecule encoded

by the canine COX-2 gene. In one embodiment, the concentration of the mRNA molecule is assayed by incubating the biological sample in the presence of a nucleic acid molecule that hybridizes to the mRNA molecule to produce a hybridized mRNA molecule, and detecting the hybridized mRNA molecule.

In another embodiment the present invention provides a method for diagnosing a COX-2 related condition in a dog which comprises the steps of: (A) incubating under conditions permitting a nucleic acid hybridization a marker nucleic acid molecule with a complementary nucleic acid molecule obtained from a biological sample from the dog; (B) hybridizing the marker nucleic acid molecule with the complementary nucleic acid molecule; and (C) assaying the hybridized marker nucleic acid molecule and complementary nucleic acid molecule for a polymorphism; thereby diagnosing the COX-2 related condition in the dog wherein: the marker nucleic acid molecule comprises a nucleotide sequence of a polynucleotide that specifically hybridizes to another polynucleotide that is linked to a canine COX-2 gene; and the polymorphism is predictive of a mutation affecting the canine COX-2 condition in the dog. Preferably, the assay detects the concentration of the hybridized marker nucleic acid molecule and complementary nucleic acid molecule. Alternatively, the assay can detect the pattern of the hybridized marker nucleic acid molecule and complementary nucleic acid molecule. In one embodiment, the marker nucleic acid molecule is a mRNA molecule encoded by the gene or a cDNA molecule encoded by the gene. The COX-2 related condition can be any condition in which the canine COX-2 protein is expressed in the animal. For example the COX-2 related condition can be inflammation, arthritis, cancer, neoplasia, a central nervous system ("CNS") disorder, an ophthalmological disorder, bronchitis, tendinitis, bursitis, skin related conditions (such as psoriasis, eczema, burns, or dermatitis), inflammatory bowel disease, gastritis, irritable bowel syndrome, Chron's disease, ulcerative colitis, vascular diseases, migraine headaches, periateritis nodosa, thyroiditis, aplastic anemia, pancreatitis, Hidgkin's disease, sclerodoma, rheumatic fever, diabetes, myasthenia gravis, sarcoidosis, nephrotic syndrome, hypertensivity, conjunctivitis, gingivitis, swelling after surgery, myocardial ischemia, and the like.

In another embodiment the present invention provides a method of determining an association between a polymorphism and a trait expressed by a dog wherein the method comprises: (A) hybridizing a nucleic acid molecule specific for the polymorphism to a gene in a cell, wherein the nucleic acid molecule has a nucleic acid sequence of SEQ ID: 3 or a complement thereof; and (B) determining the degree of association between the polymorphism and the trait. The marker nucleic acid molecule can for example comprise a nucleotide sequence of a polynucleotide that is physically linked to within 1 mb of a sequence that specifically hybridizes to the gene. The marker nucleic acid molecule can, if desired, comprise a nucleotide sequence of a polynucleotide that is physically linked to within 100 kb to a sequence that specifically hybridizes to the gene. Alternatively, the marker molecule can comprise a nucleotide sequence of a polynucleotide that is physically linked to within 10 kb to a sequence that specifically hybridizes to the gene.

In another embodiment the present invention provides a method of producing a cell containing an expressed canine COX-2 protein comprising: (A) transforming the cell with a functional nucleic acid molecule; and (B) culturing the transformed cell; thereby producing the cell containing the expressed canine COX-2 protein, wherein: the functional nucleic acid molecule comprises a promoter region, the promoter region is linked to a structural region, the structural region comprises a nucleic acid sequence of SEQ ID: 3, the structural region is linked to a 3' non-translated sequence that functions in the cell to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of a mRNA molecule; and the functional nucleic acid molecule results in expression of the canine COX-2 protein.

In another embodiment the present invention provides a method of producing a cell exhibiting co-suppression of a canine COX-2 protein comprising: (A) transforming the cell with a functional nucleic acid molecule; and (B) culturing the transformed cell; thereby producing the cell exhibiting co-suppression of the canine COX-2 protein, wherein: the functional nucleic acid molecule comprises a promoter region, the promoter region is linked to a structural region, the structural region comprises a nucleic acid sequence of SEQ ID: 3, the structural region is linked to a 3' non-translated sequence that functions in the cell to cause termination of transcription

and addition of polyadenylated ribonucleotides to a 3' end of a mRNA molecule, and the functional nucleic acid molecule results in co-suppression of the canine COX-2 protein.

In another embodiment the present invention provides a method for causing co-suppression of a canine COX-2 protein in a transformed cell comprising: (A) transforming the cell with a nucleic acid molecule to produce a transformed cell; and (B) culturing the transformed cell; thereby causing co-suppression of the canine COX-2 protein in the transformed cell, wherein: the functional nucleic acid molecule comprises a promoter region; the promoter region is linked to a structural region, the structural region comprises a nucleic acid molecule having a nucleic acid sequence of SEQ ID: 3, the structural region is linked to a 3' non-translated sequence that functions in the cell to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of a mRNA molecule, and the functional nucleic acid molecule results in co-suppression of the canine COX-2 protein.

In another embodiment the present invention provides a method of isolating a nucleic acid that encodes a canine COX-2 protein or fragment thereof comprising (A) incubating under conditions permitting nucleic acid hybridization a first nucleic acid molecule comprising SEQ ID: 3 or the complement thereof with a complementary second nucleic acid molecule obtained from a cell; (B) hybridizing the first nucleic acid molecule with the second nucleic acid molecule; and (C) isolating the second nucleic acid molecule.

In another embodiment the present invention provides a method of producing a cell containing an expressed canine COX-2 gene comprising (A) transforming the cell with a functional nucleic acid molecule; and (B) culturing the transformed cell; thereby producing the cell containing the expressed canine COX-2 gene, wherein: the functional nucleic acid molecule comprises a promoter region, the promoter region is linked to a structural region wherein the structural region comprises a nucleic acid molecule having a nucleic acid sequence consisting of SEQ ID:3 or complements thereof the structural region is linked to a 3' non-translated sequence that functions in the cell to cause termination of transcription and addition of polyadenylated

ribonucleotides to a 3' end of a mRNA molecule, and the functional nucleic acid molecule results in overexpression of the canine COX-2 gene protein.

In another embodiment the present invention provides a method of producing a cell exhibiting co-suppression of a canine COX-2 gene comprising (A) transforming the cell with a functional nucleic acid molecule; and (B) culturing the transformed cell; thereby producing a cell co-suppression of a canine COX-2 gene, wherein: the functional nucleic acid molecule comprises a promoter region, the promoter region is linked to a structural region wherein the structural region comprises a nucleic acid sequence selected from the group consisting of SEQ ID: 3 or complements thereof, the structural region is linked to a 3' non-translated sequence that functions in the cell to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of a mRNA molecule, and the functional nucleic acid molecule results in co-suppression of the canine COX-2 gene.

In another embodiment the present invention provides a method for causing co-suppression of a canine COX-2 gene in a cell comprising (A) transforming the cell with a nucleic acid molecule; and (B) culturing the transformed cell; thereby reducing expression of a canine COX-2 gene in a cell, wherein the nucleic acid molecule has an exogenous promoter region which causes the production of a mRNA molecule, the exogenous promoter region is linked to a transcribed nucleic acid molecule having a transcribed strand and a non-transcribed strand, the transcribed strand is complementary to a nucleic acid molecule having a nucleic acid selected from the group consisting of SEQ ID: 3 or complements thereof and the transcribed strand is complementary to an endogenous mRNA molecule, and the transcribed nucleic acid molecule is linked to a 3' non-translated sequence that functions in the cell to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of a mRNA molecule.

In another embodiment the present invention provides a substantially-purified nucleic acid molecule comprising a sequence which encodes a mammalian canine COX-2 protein. Preferably the substantially-purified nucleic acid encodes a sequence selected from the group consisting of human, rat, or mouse canine COX-2 protein.

More preferably it encodes a human canine COX-2 protein. More preferably still it encodes a mouse canine COX-2.

In another embodiment the present invention provides a method of identifying a test material which has the ability to inhibit, suppress, modulate, or maintain canine COX-2 activity wherein the method comprises (A) contacting a test transformed cell with the test material, wherein the transformed cell expresses a gene encoding canine COX-2, and wherein the contacting is performed in the presence of test amount of arachadonic acid; (B) measuring the conversion of prostaglandin precursor to prostaglandin product by the test transformed cells; (C) contacting a control transformed cell with a control amount of prostaglandin precursor, wherein the control transformed cell expresses a gene encoding canine COX-2, and wherein the contacting is performed substantially in the absence of the test material; (D) measuring the conversion of prostaglandin precursor to prostaglandin product by the control transformed cells; and (E) comparing the amount of prostaglandin precursor converted to prostaglandin product by the test transformed cells to the amount of prostaglandin precursor converted to prostaglandin product by the control transformed cells.

In another embodiment the present invention provides a method of identifying a test material which has the ability to inhibit, suppress, modulate, or maintain canine COX-2 activity wherein the method comprises (A) contacting a test canine COX-2 protein with the test material, wherein the contacting is performed in the presence of test amount of a prostaglandin precursor; (B) measuring the conversion of the prostaglandin precursor to a prostaglandin product by the test transformed cells; (C) contacting a control canine COX-2 protein with a control amount of the prostaglandin precursor, wherein the contacting is performed substantially in the absence of the test material; (D) measuring the conversion of the prostaglandin precursor to the prostaglandin product by the canine COX-2 protein; and (E) comparing the amount of

the prostaglandin precursor converted to prostaglandin product by the test canine COX-2 protein to the amount of prostaglandin precursor converted to prostaglandin product by the control canine COX-2 protein.

In another embodiment the present invention provides a method of identifying a test material which has the ability to inhibit, suppress, modulate, or maintain canine COX-2 activity wherein the method comprises (A) contacting a test canine COX-2 fragment with the test material, wherein the contacting is performed in the presence of test amount of a prostaglandin precursor; (B) measuring the conversion of the prostaglandin precursor to a prostaglandin product by the test transformed cells; (C) contacting a control canine COX-2 amino acid fragment with a control amount of the prostaglandin precursor, wherein the contacting is performed substantially in the absence of the test material; (D) measuring the conversion of the prostaglandin precursor to the prostaglandin product by the canine COX-2 amino acid fragment; and (E) comparing the amount of the prostaglandin precursor converted to prostaglandin product by the test canine COX-2 amino acid fragment to the amount of prostaglandin precursor converted to prostaglandin product by the control canine COX-2 amino acid fragment.

In a preferred embodiment, the prostaglandin precursor is arachadonic acid.

Having now generally described the invention, the same will be more readily understood through reference to the following examples which are provided by way of illustration, and are not intended to be limiting of the present invention, unless specified.

Example 1

cDNA Isolation

For construction of cDNA libraries for cloning canine COX-1 and COX-2, mRNA was prepared from canine kidney papillae and lipopolysaccharide (LPS)-stimulated canine mononuclear cells.

For the kidney cDNA library construction, total RNA was isolated from 200 micrograms (ug) of kidney tissue using 12 ml of TRIzol reagent (Life Technologies). RNA was prepared using the standard protocol provided by the manufacturer (Life Technologies). The protocol yielded about 200 ug of total RNA. The mRNA was then isolated using a Mini Oligo(dT) Cellulose Spin Column Kit (5 prime-3 prime). The yield was about 7 ug after a single oligo(dT) column purification.

For construction of a mononuclear cell cDNA library, mRNA was isolated directly from about 10^7 cells using the Poly(A) Pure mRNA Purification Kit (Ambion). After a single oligo(dT) purification step, 2 ug of mRNA were isolated.

Example 2

Gene Expression

Canine COX-1 was expressed using a full-length COX-1 cDNA containing the entire coding region, which was digested with SalI and NotI to generate ca.2600 base pair fragment for ligation into an insect cell expression vector, pFastBac 1 (Invitrogen), which had been digested with SalI and NotI. The resulting plasmid, pMON37775, was sequenced to confirm cloning.

An insect cell expression plasmid for canine COX-2 was created by ligating a COX-2 fragment generated by PCR fragment to a partial COX-2 cDNA. The 5' end of COX-2 was cloned from kidney double-stranded cDNA using PCR. Both fragments were ligated into pFastBack 1, which had been digested with EcoRI and NotI. The resulting plasmid, pMON37776, was confirmed by sequencing.

Example 3

Selective COX-2 Inhibitor Screening Assay

The standard screening assay for inhibitor potency (IC50) was an ELISA method for detection of PGE2. IC50 values were determined by pre-incubation of the inhibitors with enzyme for 10 min. at 25 C in a buffer containing 50 mM potassium phosphate, pH 7.5, 2 mM phenol, 1 mM heme, and 300 mM epinephrine. Inhibitors were first made as a 10 mM stock in DMSO and further diluted into 1:9 (DMSO:ethanol). The reaction was allowed to proceed for 10 min. For time dependent inhibition, the initial rate of the reaction was measured by stopping the reaction at 15 sec. with the addition of 1/10 volume N HCl; the amount of prostaglandin formed was measured by ELISA (Cayman Chemical Co., Ann Arbor, MI). IC50s were determined from a 4 parameter log fit of the data using Grafit.

All references, patents or applications cited herein are incorporated by reference in their entirety as if written herein.

We claim:

1. A substantially-purified nucleic acid molecule selected from the group of DNAs consisting of a nucleic acid molecule having SEQ ID: 1, the complement of the nucleic acid molecule having SEQ ID:1, an internal fragment of the nucleic acid molecule having SEQ ID: 1, and an internal fragment of the complement of the nucleic acid molecule having SEQ ID: 1, wherein SEQ ID: 1 encodes a canine COX-1 protein or a fragment thereof.

2. The substantially-purified nucleic acid molecule according to claim 1, wherein the substantially-purified nucleic acid molecule encodes the canine COX-1 protein.

3. The substantially-purified nucleic acid molecule according to claim 1, wherein the substantially-purified nucleic acid molecule has SEQ ID: 1 or a complement thereof.

4. The substantially-purified nucleic acid molecule according to claim 1, wherein the nucleic acid molecule specifically hybridizes to another nucleic acid molecule that encodes canine COX-1 or complement thereof and fails to specifically hybridize to a nucleic acid molecule that encodes non-canine COX-1 or complement thereof.

5. The substantially-purified nucleic acid molecule according to claim 4, wherein the nucleic acid molecule specifically hybridizes to another nucleic acid molecule that encodes canine COX-1 or complement thereof under high stringency conditions and fails to specifically hybridize to a nucleic acid

molecule that encodes non-canine COX-1 or complement thereof under high stringency conditions.

6. A substantially-purified canine COX-1 nucleic acid molecule which comprises a nucleic acid sequence that is identical to at least 20 contiguous nucleotides of SEQ ID: 1 or a complement thereof.

7. The substantially-purified canine COX-1 nucleic acid molecule according to claim 6, wherein the nucleic acid molecule comprises a nucleic acid sequence that is identical to at least 50 contiguous nucleotides of SEQ ID: 1 or a complement thereof.

8. The substantially-purified canine COX-1 nucleic acid molecule according to claim 7, wherein the nucleic acid molecule comprises a nucleic acid sequence that is identical to at least 100 contiguous nucleotides of SEQ ID: 1 or a complement thereof.

9. A substantially-purified canine COX-1 protein or a fragment thereof wherein the canine COX-1 protein or the fragment is encoded by a nucleic acid sequence which specifically hybridizes to SEQ ID: 1 or a complement thereof.

10. A substantially-purified canine COX-1 protein or a fragment thereof according to claim 9, where the nucleic acid sequence is selected from SEQ ID: 1 or a complement thereof.

11. A substantially-purified canine COX-1 protein having SEQ ID: 2.

12. A substantially-purified canine COX-1 protein or a fragment thereof comprising at least 10 consecutive amino acids of SEQ ID: 2.

13 The substantially-purified canine COX-1 protein or a fragment thereof according to claim 12, wherein the canine COX-1 protein or fragment is a fusion protein.

14. An antibody capable of specifically binding to a substantially-purified canine COX-1 protein having SEQ ID: 2.

15. The antibody of claim 14, wherein the antibody is detectably labeled.

16. A substantially-purified nucleic acid molecule selected from the group of DNAs consisting of a nucleic acid molecule having SEQ ID: 3, a complement molecule of a nucleic acid molecule having SEQ ID: 3, an internal fragment a nucleic acid molecule having SEQ ID: 3, and an internal fragment of a complement molecule of a nucleic acid molecule having SEQ ID: 3, wherein the SEQ ID: 3 encodes a canine COX-2 protein or a fragment thereof.

17. The substantially-purified nucleic acid molecule according to claim 16, wherein the substantially-purified nucleic acid molecule encodes the canine COX-2 protein.

18. The substantially-purified nucleic acid molecule according to claim 16, wherein the substantially-purified nucleic acid molecule has the sequence described by SEQ ID: 3 or a complement thereof.

19. The substantially-purified nucleic acid molecule according to claim 16, wherein the substantially-purified nucleic acid molecule specifically hybridizes to another nucleic acid molecule that encodes canine COX-2 or complement thereof and fails to specifically hybridize to a nucleic acid molecule that encodes non-canine COX-2 or complement thereof.

20. The substantially-purified nucleic acid molecule according to claim 19, wherein the substantially-purified nucleic acid molecule specifically hybridizes to another nucleic acid molecule that encodes canine COX-2 or complement thereof under high stringency conditions and fails to specifically hybridize to a nucleic acid molecule that encodes non-canine COX-2 or complement thereof under high stringency conditions.

21. A substantially-purified canine COX-2 nucleic acid molecule which comprises a nucleic acid sequence that is identical to at least 20 contiguous nucleotides of SEQ ID: 3 or a complement thereof.

22. The substantially-purified canine COX-2 nucleic acid molecule according to claim 21, wherein the nucleic acid molecule comprises a nucleic acid sequence that is identical to at least 50 contiguous nucleotides of SEQ ID: 3 or a complement thereof.

23. The substantially-purified canine COX-2 nucleic acid molecule according to claim 22, wherein the nucleic acid molecule comprises a nucleic acid sequence that is identical to at least 100 contiguous nucleotides of SEQ ID: 3 or its complement.

24. A substantially-purified canine COX-2 protein or a fragment thereof encoded by a nucleic acid sequence which specifically hybridizes to SEQ ID: 4 or its complement.

25. The substantially-purified canine COX-2 protein or a fragment thereof according to claim 24, where the nucleic acid sequence is selected from the group consisting of SEQ ID: 4 and the complement of SEQ ID: 4.

26. A substantially-purified canine COX-2 protein having SEQ ID: 4.

27. A substantially-purified canine COX-2 protein or a fragment thereof wherein the substantially-purified canine COX-2 protein or the fragment thereof comprises at least 10 consecutive amino acids of SEQ ID: 4.

28 The substantially-purified canine COX-2 protein or fragment thereof according to claim 27, wherein the canine COX-2 protein or fragment thereof is a fusion protein.

29. An antibody capable of specifically binding to the substantially-purified canine COX-2 protein having SEQ ID: 4.

30. The antibody of claim 29, wherein the antibody is detectably labeled.

31. A transformed cell having a nucleic acid molecule which comprises an exogenous promoter region linked to a structural nucleic acid sequence, and a 3'non-translated sequence linked to the structural nucleic acid sequence, wherein:

the exogenous promoter region causes production of a mRNA molecule;

the structural nucleic acid sequence encodes a canine COX-1 gene or a fragment thereof; and

the 3' non-translated sequence causes termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of the mRNA molecule.

32. The transformed cell according to claim 31, wherein the structural nucleic acid sequence is in the antisense orientation.

33. A transformed cell having a nucleic acid molecule which comprises an exogenous promoter region linked to a structural nucleic acid sequence, and a a 3' non-translated sequence, wherein:

the exogenous promoter region causes production of a mRNA molecule;

the structural nucleic acid molecule encodes a canine COX-2 gene or a fragment thereof; and

the 3' non-translated sequence causes termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of the mRNA molecule.

34. The transformed cell according to claim 33, wherein the structural nucleic acid sequence is in the antisense orientation.

35. A method for assaying a canine COX-2 protein in a cell comprising:

(A) incubating a marker nucleic acid molecule with a complementary nucleic acid molecule obtained from the cell, wherein:
the incubation is performed under conditions permitting nucleic acid hybridization; and
the marker nucleic acid molecule is capable of specifically hybridizing under high stringency conditions to a canine COX-2-encoding nucleic acid molecule or a complement thereof;

(B) hybridizing the marker nucleic acid molecule with the complementary nucleic acid molecule; and

(C) assaying the complementary nucleic acid, wherein the assay is predictive of the level or pattern of the canine COX-2 protein.

36. The method of claim 35, wherein the assay is predictive of the canine COX-2 protein concentration.

37. The method of claim 35, wherein the assay is predictive of the canine COX-2 protein pattern.

38. The method of claim 35, wherein the assay is performed by *in situ* hybridization.

39. A method for diagnosis or prognosis of a COX-2 related condition in a dog wherein the method comprises assaying the concentration of a molecule, the concentration being dependent upon the expression of a canine COX-2 gene, and the molecule being assayable in a biological sample from the dog.

40. The method of claim 39, wherein the biological sample is selected from the group consisting of skeletal tissue, blood, lymph and serum.

41. The method of claim 39, wherein the molecule is a protein molecule expressed by the canine COX-2 gene.

42. The method of claim 39, wherein the molecule is a mRNA molecule encoded by the canine COX-2 gene, or a cDNA molecule encoded by the canine COX-2 gene.

43. The method of claim 42, wherein the concentration of the mRNA molecule is assayed by incubating thebiological sample in the presence of a nucleic acid molecule that hybridizes to the mRNA molecule to produce a hybridized mRNA molecule, and detecting the hybridized mRNA molecule.

44. A method for diagnosing a COX-2 related condition in a dog which comprises the steps of:

(A) incubating under conditions permitting a nucleic acid hybridization a marker nucleic acid molecule with a complementary nucleic acid molecule obtained from a biological sample from the dog;

(B) hybridizing the marker nucleic acid molecule with the complementary nucleic acid molecule; and

(C) assaying the hybridized marker nucleic acid molecule and complementary nucleic acid molecule for a polymorphism;

thereby diagnosing the COX-2 related condition in the dog, wherein:

the marker nucleic acid molecule comprises a nucleotide sequence of a polynucleotide that specifically hybridizes to another polynucleotide that is linked to a canine COX-2 gene; and

the polymorphism is predictive of a mutation affecting the canine COX-2 condition in the dog.

45. The method of claim 44, wherein the assay detects the concentration of the hybridized marker nucleic acid molecule and complementary nucleic acid molecule .

46. The method of claim 44, wherein the assay detects the pattern of the hybridized marker nucleic acid molecule and complementary nucleic acid molecule .

47. The method of claim 44, wherein the marker nucleic acid molecule is a mRNA molecule encoded by the canine COX-2 gene or a cDNA molecule encoded by the canine COX-2 gene.

48. The method of claim 44, wherein the COX-2 related condition is inflammation.

49. The method of claim 44, wherein the COX-2 related condition is arthritis.

50. The method of claim 44, wherein the COX-2 related condition is cancer.

51. The method of claim 44, wherein the COX-2 related condition is neoplasia.

52. The method of claim 44, wherein the COX-2 related condition is a CNS disorder.

53. A method of determining an association between a polymorphism and a trait wherein the method comprises:

(A) hybridizing a nucleic acid molecule specific for the polymorphism to a gene in a cell, wherein the nucleic acid molecule has a nucleic acid sequence of SEQ ID: 3 or a complement thereof; and

(B) determining the degree of association between the polymorphism and the trait.

54. The method of claim 53, wherein the marker nucleic acid molecule comprises a nucleotide sequence of a polynucleotide that is physically linked to within 1 mb of a sequence that specifically hybridizes to the gene.

55. The method of claim 53, wherein the marker nucleic acid molecule comprises a nucleotide sequence of a polynucleotide that is physically linked to within 100 kb to a sequence that specifically hybridizes to the gene.

56. The method of claim 53, wherein the marker nucleic acid molecule comprises a nucleotide sequence of a polynucleotide that is physically linked to within 10 kb to a sequence that specifically hybridizes to the gene.

57. A method of producing a cell containing an expressed canine COX-2 protein comprising:

(A) transforming the cell with a functional nucleic acid molecule; and

(B) culturing the transformed cell;

thereby producing the cell containing the expressed canine COX-2 protein, wherein:

the functional nucleic acid molecule comprises a promoter region;

the promoter region is linked to a structural region;

the structural region comprises a nucleic acid sequence of SEQ ID: 3,

the structural region is linked to a 3' non-translated sequence that functions in the cell to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of a mRNA molecule; and

the functional nucleic acid molecule results in expression of the canine COX-2 protein.

58. A method of producing a cell exhibiting co-suppression of a canine COX-2 protein comprising:

(A) transforming the cell with a functional nucleic acid molecule; and

(B) culturing the transformed cell;

thereby producing the cell exhibiting co-suppression of the canine COX-2 protein, wherein:

the functional nucleic acid molecule comprises a promoter region;

the promoter region is linked to a structural region;

the structural region comprises a nucleic acid sequence of SEQ ID: 3;

the structural region is linked to a 3' non-translated sequence that functions in the cell to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of a mRNA molecule; and

the functional nucleic acid molecule results in co-suppression of the canine COX-2 protein.

59. A method for causing co-suppression of a canine COX-2 protein in a transformed cell comprising:

(A) transforming the cell with a nucleic acid molecule to produce a transformed cell; and

(B) culturing the transformed cell;

thereby causing co-suppression of the canine COX-2 protein in the transformed cell, wherein:

the functional nucleic acid molecule comprises a promoter region;

the promoter region is linked to a structural region;

the structural region comprises a nucleic acid molecule having a nucleic acid sequence of SEQ ID: 3;

the structural region is linked to a 3' non-translated sequence that functions in the cell to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of a mRNA molecule; and

the functional nucleic acid molecule results in co-suppression of the canine COX-2 protein.

60. A method of isolating a nucleic acid molecule that encodes a canine COX-2 protein or a fragment thereof comprising:

(A) incubating under conditions permitting nucleic acid hybridization a first nucleic acid molecule comprising SEQ ID: 3 or the complement thereof with a complementary second nucleic acid molecule obtained from a cell;

(B) hybridizing the first nucleic acid molecule with the second nucleic acid molecule; and

(C) isolating the second nucleic acid molecule.

61. A method of producing a cell containing an expressed canine COX-2 gene comprising:

(A) transforming the cell with a functional nucleic acid molecule; and

(B) culturing the transformed cell.

thereby producing the cell containing the expressed canine COX-2 gene, wherein:

the functional nucleic acid molecule comprises a promoter region;

the promoter region is linked to a structural region wherein the structural region comprises a nucleic acid molecule having a nucleic acid sequence consisting of SEQ ID:3 or complements thereof;

the structural region is linked to a 3' non-translated sequence that functions in the cell to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of a mRNA molecule; and

the functional nucleic acid molecule results in overexpression of the canine COX-2 gene protein.

62. A method of producing a cell exhibiting co-suppression of a canine COX-2 gene comprising:

(A) transforming the cell with a functional nucleic acid molecule; and

(B) culturing the transformed cell;

thereby producing a cell co-suppression of a canine COX-2 gene, wherein:

the functional nucleic acid molecule comprises a promoter region;

the promoter region is linked to a structural region wherein the structural region comprises a nucleic acid sequence selected from the group consisting of SEQ ID: 3 or complements thereof;

the structural region is linked to a 3' non-translated sequence that functions in the cell to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of a mRNA molecule; and

the functional nucleic acid molecule results in co-suppression of the canine COX-2 gene.

63. A method for causing co-suppression of a canine COX-2 gene in a cell comprising:

(A) transforming the cell with a nucleic acid molecule; and

(B) culturing the transformed cell;

thereby reducing expression of a canine COX-2 gene in a cell, wherein:

the nucleic acid molecule has an exogenous promoter region which functions in a cell to cause the production of a mRNA molecule;

the exogenous promoter region is linked to a transcribed nucleic acid molecule having a transcribed strand and a non-transcribed strand;

the transcribed strand is complementary to a nucleic acid molecule having a nucleic acid selected from the group consisting of SEQ ID: 3 or complements thereof and the transcribed strand is complementary to an endogenous mRNA molecule; and

the transcribed nucleic acid molecule is linked to a 3' non-translated sequence that functions in the cell to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of a mRNA molecule.

64. A method of identifying a test material which has the ability to inhibit, suppress, modulate, or maintain canine COX-2 activity wherein the method comprises:

(A) contacting a test transformed cell with the test material, wherein the test transformed cell expresses a gene encoding canine COX-2, and wherein the contacting is performed in the presence of test amount of a prostaglandin precursor;

(B) measuring the conversion of prostaglandin precursor to a prostaglandin product by the test transformed cell;

(C) contacting a control transformed cell with a control amount of the prostaglandin precursor, wherein the control transformed cell expresses a gene encoding canine COX-2, and wherein the contacting is performed substantially in the absence of the test material;

(D) measuring the conversion of the prostaglandin precursor to prostaglandin product by the control transformed cell; and

(E) comparing the amount of the prostaglandin precursor converted to prostaglandin product by the test transformed cell to the amount of prostaglandin precursor converted to prostaglandin product by the control transformed cell, thereby identifying the test material.

65. A method of identifying a test material which has the ability to inhibit, suppress, modulate, or maintain canine COX-2 activity wherein the method comprises:

(A) contacting a canine COX-2 protein with the test material, and wherein the contacting is performed in the presence of test amount of a prostaglandin precursor;

(B) measuring the conversion of the prostaglandin precursor to a prostaglandin product;

(C) contacting a control canine COX-2 protein with a control amount of the prostaglandin precursor, and wherein the contacting is performed substantially in the absence of the test material;

(D) measuring the conversion of the prostaglandin precursor to prostaglandin product; and

(E) comparing the amount of the prostaglandin precursor converted to prostaglandin product by the canine COX-2 protein to the amount of the prostaglandin precursor converted to prostaglandin product by the control canine COX-2 protein, thereby identifying the test material.

66. A method of identifying a test material which has the ability to inhibit, suppress, modulate, or maintain canine COX-2 activity wherein the method comprises:

(A) contacting a canine COX-2 amino acid fragment with the test material, and wherein the contacting is performed in the presence of test amount of a prostaglandin precursor;

(B) measuring the conversion of the prostaglandin precursor to a prostaglandin product;

(C) contacting a control canine COX-2 amino acid fragment with a control amount of the prostaglandin precursor, and wherein the contacting is performed substantially in the absence of the test material;

(D) measuring the conversion of the prostaglandin precursor to prostaglandin product; and

(E) comparing the amount of the prostaglandin precursor converted to prostaglandin product by the canine COX-2 amino acid fragment to the amount of the prostaglandin precursor converted to prostaglandin product by the control canine COX-2 amino acid fragment, thereby identifying the test material.

67. The method of claims 64, 65, or 66 wherein the prostaglandin precursor is arachadonic acid.

```
CANINE  MSRGSRLHRW PLLL--LL-L PLLLPPPVLP AE--ARTPAP VNPCCYYPCQ
        ::: :      :::  :: : ::::: :::: :.  : :  : ::::::::::
HUMAN   MSR-S----- -LLLRFLLFL LLLPPLPVLL ADPGAPT--P VNPCCYYPCQ  41

        HQGICVRFGL DRYQCDCTRT GYSGPNCTIP ELWTWLRNSL RPSPSFLHFL
        :::::::::: :::::::::: :::::::::: ::::::::: :::::: :::
        HQGICVRFGL DRYQCDCTRT GYSGPNCTIP GLWTWLRNSL RPSPSFLHFL  91

        LTHGRWFWEF INATFIRDML MRLVLTARSN LIPSPPTYNI AHDYISWESF
        :::::::::: .:::::::.: :::::: ::: :::::::::: ::::::::::
        LTHGRWFWEF VNATFIREMR MRLVLTVRSN LIPSPPTYNS AHDYISWESF 141

        SNVSYYTRVL PSVPQDCPTP MGTKGKKQLP DAQLLGRRFL LRRKFIPDPQ
        :::::::::: :::: ::::: :::::::::: ::::: :::: ::::::::::
        SNVSYYTRIL PSVPKDCPTP MGTKGKKQLP DAQLLARRFL LRRKFIPDPQ 191

        GTNLMFAFFA QHFTHQFFKT SGKMGPGFTK DAQLLGRRFL IYGDNLDRQY
        :::::::::: :::::::::: :::::::::: :::::::::: ::::::.:::
        GTNLMFAFFA QHFTHQFFKT SGKMGPGFTK DAQLLARRFL IYGDNLERQY 241

        QLRLFKDGKL KYQVLDGEMY PPSVEEAPVL MHYPRGILPQ SQMAVGQEVF
        :::::::::: :::::::::: :::::::::: ::::::: :: ::::::::::
        QLRLFKDGKL KYQVLDGEMY PPSVEEAPVL MHYPRGIPPQ SQMAVGQEVF 250

        GLLPGLMLYA TLWLREHNRV CDLLKAEHPT WDGEQLFQTA RLILIGETIK
        :::::::::: :::::::::: :::::::::: :::::::::: ::::::::::
        GLLPGLMLYA TLWLREHNRV CDLLKAEHPT WDGEQLFQTT RLILIGETIK 300

        IVIEEYVQQL SGYFLQLKFD PELLFSAQFQ YRNRIAMEFN QLYHWHPLMP
        :::::::::: :::::::::: :::::  ::: :::::::::: ::::::::::
        IVIEEYVQQL SGYFLQLKFD PELLFGVQFQ YRNRIAMEFN HLYHWHPLMP 350

        DSFWVGSQEY SYEQFLFNTS MLTHYGIEAL VDAFSRQSAG RIGGGRNIDH
        ::: :::::: :::::::::: ::  ::.::: ::::::: :: ::::::::::
        DSFKVGSQEY SYEQFLFNTS MLVDYGVEAL VDAFSRQIAG RIGGGRNMDH 400
```

fig. 1A

```
CANINE  HVLHVAVETI KESRELRLQP FNEYRKRFGM RPYMSFQELT GEKEMAAELE
        :.::::. : .::::.:::: :::::::::: .:: ::::: ::::::::::
HUMAN   HILHVAVDVI RESREMRLQP FNEYRKRFGM KPYTSFQELV GEKEMAAELE 450

        ELYGDIDALE FYPGLLLEKC HPNSIFGESM IEIGAPFSLK GLLGNPICSP
        :::::::::: :::::::::: :::::::::: :::::::::: ::::::::::
        ELYGDIDALE FYPGLLLEKC HPNSIFGESM IEIGAPFSLK GLLGNPICSP 500

        EYWKPSTFGG EMGFNMVKTA TLKKLVCLNT KTCPYVSFRV PDPHQDGGPG
        :::::::::: :.::::.:::: :::::::::: :::::::::: ::  :: ::
        EYWKPSTFGG EVGFNIVKTA TLKKLVCLNT KTCPYVSFRV PDASQDDGPA 550

         VERPSTEL
         ::::::::
         VERPSTEL
```

fig. 1B

```
CANINE  MLFRAVLLCA ALAVVRAANP CCSHPCQNQG ICMSTGFDQY KCDCTRTGFY
        :: ::.:::: ::.  .::: :::::::: : .::: ::::: ::::::::::
HUMAN   MLARALLLCA VLALSHTANP CCSHPCQNRG VCMSVGFDQY KCDCTRTGFY  50

        GENCSTPEFL TRIKLYLKPT PNTVHYILTH FKGVWNIVNN IPFLRNTIMK
        :::::::::: :::::.:::: :::::::::: ::: ::.::: ::::::::.:
        GENCSTPEFL TRIKLFLKPT PNTVHYILTH FKGFWNVVNN IPFLRNAIMS 100

        YVLTSRSHLI ESPPTYNVNY GYKSWEAFSN LSYYTRALPP VPDDCPTPMG
        :::::::::: .:::::: : :::::::::: :::: .::: ::::::::.:
        YVLTSRSHLI DSPPTYNADY GYKSWEAFSN LSYYTRALPP VPDDCPTPLG 150

        VKGKKELPDS KEIVEKFLLR RKFIPDPQGT NMMFAFFAQH FTHQFFKTDH
        ::::: :::: ::: : ::: :::::::::. ::::::::: ::::::::::
        VKGKKQLPDS NEIVGKLLLR RKFIPDPQGS NMMFAFFAQH FTHQFFKTDH 200

        KRGPAFTKGL GHGVDLNHVY GETLDRQHKL RLFKDGKMKY QVIDGEVYPP
        ::::::: :: ::::::::.: :::: :: :: :::::::::: :.::::.:::
        KRGPAFTNGL GHGVDLNHTY GETLARQRKL RLFKDGKMKY QIIDGEMYPP 250

        TVKDTQVEMI YPPHVPEHLQ FAVGQEVFGL VPGLMMYATI WLREHNRVCD
        :::::: ::: ::: ::::: :::::::::: :::::::::: ::::::::::
        TVKDTQAEMI YPPQVPEHLR FAVGQEVFGL VPGLMMYATI WLREHNRVCD 300

        VLKQEHPEWG DEQLFQTSRL ILIGETIKIV IEDYVQHLSG YHFKLKFDPE
        :::::::::: :: ::::::: :::::::::: :::::::::: ::::::::::
        VLKQEHPEWG DEQLFQTSRL ILIGETIKIV IEDYVQHLSG YHFKLKFDPE 350

        LLFNQQFQYQ NRIAAEFNTL YHWHPLLPDT LQIDDQEYNF QQFIYNNSIL
        :::: ::::: :::::::::: :::::::::  :: :: ::. ::::::::::
        LLFNKQFQYQ NRIAAEFNTL YHWHPLLPDT FQIDHQKYNY QQFIYNNSIL 400

        LEHGLTQFVE SFTRQIAGRV AGGRNVPPAV QQVSKASIDQ SRQMKYQSLN
        :::::::::: ::.::::::: ::::::: :: : :  ::::: :::::::: :
        LEHGITQFVE YESFEELTGE AGGRNVPPAV QKVSQASIDQ SRQMKYQSFN 450
```

fig. 2A

```
EYRKRFRLKP YTSFEELTGE KEMAAGLEAL YGDIDAMELY PALLVEKPRP
:::::: ::: : :::::::: :::.: :::: :::::.::: ::::::::::
EYRKRFMLKP YESFEELTGE KEMSAELEAL YGDIDAVELY PALLVEKPRP 500

EYRKRFRLKP MGAPFSLKGL MGNPICSPDY WKPSTFGGEV GFKIINTASI
:::::::::: .:::::::::: ::: :::: : :::::::::: :: :::::::
EYRKRFMLKP VGAPFSLKGL MGNVICSPAY WKPSTFGGEV GFQIINTASI 550

QSLICNNVKG CTFTAFSVQD GQLTKTVTIN ASSSHSGLDD PDPHQDGGPG
:::::::::: ::::.::: :   : :::::: :::: ::::: ::::::::::
QSLICNNVKG CTFTSFSVPD PELIKTVTIN ASSSRSGLDD PDASQDDGPA 600

STEL
::::
STEL
```

fig. 2B

```
COX2_MOUSE        MLFRAVLLCAALGLSQAANPCCSNPCQRNGECMSTGFDQYKCDCTRTGFYGENCTTPEFL
COX2_RAT          MLFRAVLLCAALALSHAANPCCSNPCQNRGECMSIGFDQYKCDCTRTGFYGENCTTPEIL
COX2_GUINEAPIG    MLARALLLCAALALGQAANPCCSNPCQNRGECLSVGFDRYKCDCTRTGYYGENCTTPEFL
COX2_RABBIT       MLARALLLCAAVALSHAANPCCSNPCQNRGVCMTMGFDQYKCDCTRTGFYGENCSTPEFL
COX2_HORSE        MLARALLLCVALALGHAANPCCSNPCQNRGVCMSVGFDQYQCDCTRTGFYGENCSTPEFL
COX2_CANINE       MLARALVLCAALAVVRAANPCCSHPCQNQGICMSTGFDQYKCDCTRTGFYGENCSTPEFL
COX2_SHEEP        MLARALLLCAAVVCG-AANPCCSHPCQNRGVCMSVGFDQYKCDCTRTGFYGENCTTPEFL
COX2_HUMAN        MLARALLLCAVLALSHTANPCCSHPCQNRGVCMSVGFDQYKCDCTRTGFYGENCTTPEFL
COX2_CHICKEN      MLLPCALLAALLAAGHAANPCCSLPCQNRGVCMTTGFDRYECDCTRTGYYGENCTTPEFF
                  **  . **.. :    :****** ****:* *:: ***:*:*******:*****:***::

COX2_MOUSE        TRIKLLLKPTPNTVHYILTHFKGVWNIVNNIPFLRSLTMKYVLTSRSYLIDSPPTYNVHY
COX2_RAT          TRIKLLLKPTPNTVHYILTHFKGVWNIVNNIPFLRNSIMRYVLTSRSHLIDSPPTYNVHY
COX2_GUINEAPIG    TRIKLLLKPTPNTVHYILTHFKGVWNIVNNIPFLRNAIMIYVLTSRSHLIDSPPTYNAHY
COX2_RABBIT       TRIKLLLKPTPDTVHYILTHFKGVWNIVNSIPFLRNSIMKYVLTSRSHMIDSPPTYNVHY
COX2_HORSE        TRIKLFLKPTPNTVHYILTHFKGVWNIVNSFPFLRNAVMKYVLVSRSHLIESPPTYNAQY
COX2_CANINE       TRIKLYLKPTPNTVHYILTHFKGVWNIVNSIPFLRNTIMKYVLTSRSHLIESPPTYNVNY
COX2_SHEEP        TRIKLLLKPTPDTVHYILTHFKGVWNIVNKISFLRNMIMRYVLTSRSHLIESPPTYNVHY
COX2_HUMAN        TWLKLILKPTPDTVHYILTHFKGFWNIINNISFLRDTIMRYVLTSRSHLIDSPPTYNSDY
COX2_CHICKEN      TWLKLILKPTPDTVHYILTHFKGVWNIINNISFLRDTIMRYVLTSRSHLIDSPPTYNSDY
                  * :** *****:**********.**::*.:.***.  * ***.***::*:****** .*
```

fig. 3A

```
COX2_MOUSE        GYKSWEAFSNLSYYTRALPPVADDCPTPMGVKGNKELPDSKEVLEKVLLRREFIPDPQGS
COX2_RAT          GYKSWEAFSNLSYYTRALPPVADDCPTPMGVKGNKELPDSKEVLEKVLLRREFIPDPQGT
COX2_GUINEAPIG    GYKSWEAFSNLSYYTRALPPVADDCPTPMGVKGKKELPDSNEVLEKVLLRRKFIPDPQGT
COX2_RABBIT       NYKSWEAFSNLSYYTRALPPVADDCPTPMGVKGKKELPDSKDVVEKLLLRRKFIPDPQGT
COX2_HORSE        GYKSWESFSNLSYYTRALPPVADGCPTPMGVKGKKELPDSKEIVEKFLLRRKFIPDPQGT
COX2_CANINE       GYKSWESFSNLSYYTRALPPVPDDCPTPMGVKGKKELPDSKEIVEKFLLRRKFIPDPQGT
COX2_SHEEP        SYKSWEAFSNLSYYTRALPPVPDDCPTPMGVKGRKELPDSKEVVKKVLLRRKFIPDPQGT
COX2_HUMAN        GYKSWEAFSNLSYYTRALPPVPDDCPTPLGVKGKKQLPDSNEIVEKLLLRRKFIPDPQGS
COX2_CHICKEN      SYKSWEAYSNLSYYTRSLPPVGHDCPTPMGVKGKKELPDSKLIVEKFLLRRKFIPDPQGT
                  .*****:********:**** ..****:****.*:****: :::*.****:*******.

COX2_MOUSE        NMMFAFFAQHFTHQFFKTDHKRGPGFTRGLGHGVDLNHIYGETLDRQHKLRLFKDGKLKY
COX2_RAT          NMMFAFFAQHFTHQFFKTDQKRGPGFTRGLGHGVDLNHVYGETLDRQHKLRLFQDGKLKY
COX2_GUINEAPIG    NMMFAFFAQHFTHQFFKSDQKRGPAFTTGLAHGVDLSHIYGETLDRQHKLRLFKDGKMKY
COX2_RABBIT       NMMFAFFAQHFTHQFFKTDLKRGPAFTKGLGHGVDLNHIYGETLDRQHKLRLFKDGKMKY
COX2_HORSE        NMMFAFFAQHFTHQFFKTDPKRGPAFTKGLGHGVDLSHIYGETLDRQHKLRLFKDGKMKY
COX2_CANINE       NMMFAFFAQHFTHQFFKTDHKRGPAFTKGLGHGVDLNHVYGETLDROHKLRLFKDGKMKY
COX2_SHEEP        NLMFAFFAQHFTHQFFKTDIERGPAFTKGKNHGVDLSHVYGESLERQHNRRLFKDGKMKY
COX2_HUMAN        NMMFAFFAQHETHQFFKTDHKRGPAFTNGLGHGVDLNHIYGETLARQRKLRLFKDGKMKY
COX2_CHICKEN      NLMFAFFAQHFTHQFFKTDIERGPAFTKGKNHGVDLSHVYGETLERQHNRRLFKDGKMKY
                  *:**:************:* ::**.** . ******.*:***:* ** : ** :***:**
```

fig. 3B

```
COX2_MOUSE       QVIGGEVYPPTVKDTQVEMIYPPHIPENLQFAVGQEVFGLVPGLMMYATIWLREHNRVCD
COX2_RAT         QVIGGEVYPPTVKDTQVDMIYPPHVPEHLRFAVGQEVFGLVPGLMMYATIWLREHNRVCD
COX2_GUINEAPIG   QIIDGEMYPPTVKETQVEMMYPPYIPEHARFAVGQEVFGLVPGLMMYATIWLREHNRVCD
COX2_RABBIT      QVIDGEVYPPTVKDTQVEMIYPPHIPAHLQFAVGQEVFGLVPGLMMYATIWLREHNRVCD
COX2_HORSE       QIINGEVYPPTVKDTQVEMIYPPHIPEHLRFAVGQEVFGLVPGLMMYATIWLREHNRVCD
COX2_CANINE      QVIDGEVYPPTVKDTQVEMIYPPHVPEHLQFAVGQEVFGLVPGLMMYATIWLREHNRVCD
COX2_SHEEP       QMINGEMYPPTVKDTQVEMIYPPHIPEHLKFAVGQEVFGLVPGLMMYATIWLREHNRVCD
COX2_HUMAN       QIIDGEMYPPTVKDTQAEMIYPPQVPEHLRFAVGQEVFGLVPGLMMYATIWLREHNRVCD
COX2_CHICKEN     QMIDGEMYPPTVKDTQAEMIYPPHVPEHLQFSVGQEVFGLVPGLMMYATIWLREHNRVCD
                 *:*.**:******:**.:*:*** :* : :*:****************************

COX2_MOUSE       ILKQEHPEWGDEQLFQTSRLILIGETIKIVIEDYVQHLSGYHFKLKFDPELLFNQQFQYQ
COX2_RAT         ILKQEHPEWDDERLFQTSRLILIGETIKIVIEDYVQHLSGYHFKLKFDPELLFNQQFQYQ
COX2_GUINEAPIG   VLKQEHPEWDDERLFQTSRLILIGETIKIVIEDYVQHLSGYHFKLKFDPELLFNQQFQYQ
COX2_RABBIT      VLKQEHPEWDDEQLFQTSRLILIGETIKIVIEDYVQHLSGYHFKLKFDPELLFNQQFQYQ
COX2_HORSE       VLKQEHPEWDDERLFQTSRLILIGETIKIVIEDYVQHLSGYHFKLKFDPELLFNQQFQYQ
COX2_CANINE      VLKQEHPEWDDERLFQTSRLILIGETIKIVIEDYVQHLSGYHFKLKFDPELLFNQQFQYQ
COX2_SHEEP       VLKQEHPEWGDEQLFQTSRLILIGETIKIVIEDYVQHLSGYHFKLKFDPELLFNQQFQYQ
COX2_HUMAN       VLKQEHPEWGDEQLFQTSRLILIGETIKIVIEDYVQHLSGYHFKLKFDPELLFNKQFQYQ
COX2_CHICKEN     VLKQEHPEWGDEQLFQTSRLILIGETIKIVIEDYVQHLSGYHFKLKFDPELLFNKQFQYQ
                 :********.**:****:**********************************:*****
```

fig. 3C

```
COX2_MOUSE       NRIASEFNTLYHWHPLLPDTENIEDQEYSFKQFLYNNSILLEHGLTQFVESFTRQIAGRV
COX2_RAT         NRIASEFNTLYHWHPLLPDTENIEDQEYTFKQFLYNNSILLEHGLAHFVESFTRQIAGRV
COX2_GUINEAPIG   NRIASEFNTLYHWHPLLPDTFQIDDQVYNFKQFLYNNSILLEHGLAHFVESFTRQIAGRV
COX2_RABBIT      NRIAAEFNTLYHWHPLLPDTFQIDDQQYNYQQFLYNNSILLEHGLTQFVESFTRQIAGRV
COX2_HORSE       NRIAAEFNTLYHWHPLLPDTFQIDDQEYNFQQFLYNNSILVEHGLTQFVESFSRQIAGRV
COX2_CANINE      NRIAAEFNTLYHWHPLLPDTLQIDDQEYNFQQFIYNNSILLEHGLTQFVESFSRQIAGRV
COX2_SHEEP       NRIAAEFNTLYHWHPLLPDVFQIDGQEYNYQQFIYNNSVLLEHGVTQFVESETRQIAGRV
COX2_HUMAN       NRIAAEFNTLYHWHPLLPDTFQIHDQKYNYQQFIYNNSILLEHGITQFVESFTRQIAGRV
COX2_CHICKEN     NRIAAEFNTLYHWHPLLPDTFQIHNQEYTFQQFLYNNSIMLEHGLSHMVKSFSKQSAGRV
                 ****:**************.::*..* *.::**:****:::***::::*:**::* ****

COX2_MOUSE       AGGRNVPIAVQAVAKASIDQSREMKYQSLNEYRKRFSLKPYTSFEELTGEKEMAAELKAL
COX2_RAT         AGGRNVPIAVQAVAKASIDQSREMKYQSLNEYRKRFSLKPYTSFEELTGEKEMAAELKAL
COX2_GUINEAPIG   AGGRNVPLAVQRVAKASIEHSRKMKYQSLNEYRKRFLMKPYTSFEELTGEKEMAAGLEAL
COX2_RABBIT      AGGRNVPPAVQKVAKASIDQSRQMKYQSLNEYRKRFLLKPYESFEELTGEKEMAAELEAL
COX2_HORSE       AGGRNVPAAAQKIAKASIDQSREMKYQSLNEYRKRFRLTPYKSFEELTGEKEMAAELEAL
COX2_CANINE      AGGRNVPAAVQQVAKASIDQSRQMKYQSLNEYRKRFRLKPYTSFEELTGEKEMAAGLEAL
COX2_SHEEP       AGRRNLPAAVEKVSKASLDQSREMKYQSFNEYRKRFLLKPYESFEELTGEKEMAAELEAL
COX2_HUMAN       AGGRNVPPAVQKVSQASIDQSRQMKYQSFNEYRKRFMLKPYESFEELTGEKEMSAELEAL
COX2_CHICKEN     AGGKNVPWAVQKVAKASIDQSRQMRYQSLNEYRKRFMLKPFKSFEELTGEKEMAAELEEL
                 ** :*:* *.: :::**:::**:*:***:******** :.*: ***********:* *: *
```

fig. 3D

```
COX2_MOUSE       YSDIDVMELYPALLVEKPRPDAIFGETMVELGAPFSLKGLMGNPICSPQYWKPSTFGGEV
COX2_RAT         YHDIDAMELYPALLVEKPRPDAIFGETMVELGAPFSLKGLMGNPICSPQYWKPSTFGGEV
COX2_GUINEAPIG   YGDIDAMELYPALLVEKPRPDAIFGETMVEMGAPFSLKGLMGNPICSPHYWKPSTFGGEV
COX2_RABBIT      YGDIDAVELYPALLVERPRPDAIFGESMVEMGAPFSLKGLMGNPICSPNYWKPSTFGGEV
COX2_HORSE       YGDIDAMELYPALLVEKPRPDAIFGETMVELGAPFSLKGLLGNPICSPDYWKPSTFGGEV
COX2_CANINE      YGDIDAMELYPALLVEKPRPDAIFGETMVEMGAPFSLKGLMGNPICSPDYWKPSTFGGEV
COX2_SHEEP       YGDIDAMELYPALLVEKPAPDAIFGETMVEAGAPFSLKGLMGNPICSPEYWKPSTFGGEV
COX2_HUMAN       YGDIDAMELYPGLLVEKPRPGAIFGETMVEIGAPFSLKGLMGNTICSPEYWKPSTFGGKV
COX2_CHICKEN     YGDIDAMELYPGLLVEKPRPGAIFGETMVEIGAPFSLKGLMGNTICSPEYWKPSTFGGKV
                 * ***.:****.****:* *.*****:*** *********:** **** *********:*

COX2_MOUSE       GFKIINTASIQSLICNNVKGCPFTSFNVQDPQPTKTATINASASHSRLDDINPTVLIKRR
COX2_RAT         GFRIINTASIQSLICNNVKGCPFTSFNVQDPQPTKTATINASASHSRLDDINPTVLIKRR
COX2_GUINEAPIG   GFQIVNTASIQSLICNNVKGCPVTAFNLPDPQLAKTVTINASASHSRLEDLSPTVLLKGR
COX2_RABBIT      GFKIVNTASIQSLICNNVKGCPFTSFNVPDPQLTKTVTINASASHSRLEDINPTVLLKGR
COX2_HORSE       GFKIINTASIQSLICNNVKGCPFTAFSVQDPQLSKAVTINASASHSGLDDVNPTVLLKER
COX2_CANINE      GFKIINTASIQSLICNNVKGCPFTAFSVQDGQLTKTVTINASSSHSGLDDINPTVLLKER
COX2_SHEEP       GFKIINTASIQSLICSNVKGCPETSFSVQDAHLTKTVTINASSSHSGLDDINPTVLLKER
COX2_HUMAN       GFQIINTASIQSLICNNVKGCPFTSFSVPDPELIKTVTINASSSRSGLDDINPTVLLKEQ
COX2_CHICKEN     GFEIINTASLQKLICNNVKGCPFTAFHVLNPEPT-EATINVSTSNTAMEDINPTLLLKEQ
                 **.*:****:*.***.******.::*: :  .    .***.*:*.: ::*:.**:*:* :
```

fig. 3E

```
COX2_MOUSE      STEL
COX2_RAT        STEL
COX2_GUINEAPIG  STEL
COX2_RABBIT     STEL
COX2_HORSE      STEL
COX2_CANINE     STEL
COX2_SHEEP      STEL
COX2_HUMAN      STEL
COX2_CHICKEN    STEL
                *:**
```

fig. 3F

<110> Gierse, James K.

<120> Canine Cyclooxygenase-1 (COX-1) and Cyclooxygenase-2 (COX-2)

<150> US 60/147,601

<160> 15

<210> 1
<211> 2587
<212> DNA
<213> Canis familiaris

<400> 1

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gaacccgtgt tgttactacc catgtcagca ccaagggatc tgtgtccgct tcggccttga 180

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1560

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1620

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2220

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2340

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2400

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2580

ttttccc
2587

<210> 2
<211> 608
<212> PRT
<213> Canis familiaris

<223> unsure at all Xaa locations
<400> 2

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1 5 10 15

Leu Leu Xaa Leu Leu Leu Leu Pro Pro Pro Pro Val Leu Pro Ala Glu
20 25 30

Xaa Xaa Ala Arg Thr Pro Ala Pro Val Asn Pro Cys Cys Tyr Tyr Pro
35 40 45

Cys Gln His Gln Gly Ile Cys Val Arg Phe Gly Leu Asp Arg Tyr Gln

```
     50                  55                  60

Cys Asp Cys Thr Arg Thr Gly Tyr Ser Gly Pro Asn Cys Thr Ile Pro
 65                  70                  75                  80

Glu Leu Trp Thr Trp Leu Arg Asn Ser Leu Arg Pro Ser Pro Ser Phe
                 85                  90                  95

Leu His Phe Leu Leu Thr His Gly Arg Trp Phe Trp Glu Phe Ile Asn
            100                 105                 110

Ala Thr Phe Ile Arg Asp Met Leu Met Arg Leu Val Leu Thr Ala Arg
        115                 120                 125

Ser Asn Leu Ile Pro Ser Pro Pro Thr Tyr Asn Ile Ala His Asp Tyr
    130                 135                 140

Ile Ser Trp Glu Ser Phe Ser Asn Val Ser Tyr Tyr Thr Arg Val Leu
145                 150                 155                 160

Pro Ser Val Pro Gln Asp Cys Pro Thr Pro Met Gly Thr Lys Gly Lys
                165                 170                 175

Lys Gln Leu Pro Asp Ala Gln Leu Leu Gly Arg Arg Phe Leu Leu Arg
            180                 185                 190

Arg Lys Phe Ile Pro Asp Pro Gln Gly Thr Asn Leu Met Phe Ala Phe
        195                 200                 205

Phe Ala Gln His Phe Thr His Gln Phe Phe Lys Thr Ser Gly Lys Met
    210                 215                 220

Gly Pro Gly Phe Thr Lys Ala Leu Gly His Gly Val Asp Leu Gly His
225                 230                 235                 240

Ile Tyr Gly Asp Asn Leu Asp Arg Gln Tyr Gln Leu Arg Leu Phe Lys
                245                 250                 255

Asp Gly Lys Leu Lys Tyr Gln Val Leu Asp Gly Glu Met Tyr Pro Pro
            260                 265                 270

Ser Val Glu Glu Ala Pro Val Leu Met His Tyr Pro Arg Gly Ile Leu
        275                 280                 285

Pro Gln Ser Gln Met Ala Val Gly Gln Glu Val Phe Gly Leu Leu Pro
    290                 295                 300

Gly Leu Met Leu Tyr Ala Thr Leu Trp Leu Arg Glu His Asn Arg Val
305                 310                 315                 320

Cys Asp Leu Leu Lys Ala Glu His Pro Thr Trp Gly Asp Glu Gln Leu
                325                 330                 335

Phe Gln Thr Ala Arg Leu Ile Leu Ile Gly Glu Thr Ile Lys Ile Val
            340                 345                 350
```

Ile Glu Glu Tyr Val Gln Gln Leu Ser Gly Tyr Phe Leu Gln Leu Lys
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Phe Asp Pro Glu Leu Leu Phe Ser Ala Gln Phe Gln Tyr Arg Asn Arg
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Ile Ala Met Glu Phe Asn Gln Leu Tyr His Trp His Pro Leu Met Pro
385 390 395 400

Asp Ser Phe Trp Val Gly Ser Gln Glu Tyr Ser Tyr Glu Gln Phe Leu
405 410 415

Phe Asn Thr Ser Met Leu Thr His Tyr Gly Ile Glu Ala Leu Val Asp
420 425 430

Ala Phe Ser Arg Gln Ser Ala Gly Arg Ile Gly Gly Gly Arg Asn Ile
435 440 445

Asp His His Val Leu His Val Ala Val Glu Thr Ile Lys Glu Ser Arg
450 455 460

Glu Leu Arg Leu Gln Pro Phe Asn Glu Tyr Arg Lys Arg Phe Gly Met
465 470 475 480

Arg Pro Tyr Met Ser Phe Gln Glu Leu Thr Gly Glu Lys Glu Met Ala
485 490 495

Ala Glu Leu Glu Glu Leu Tyr Gly Asp Ile Asp Ala Leu Glu Phe Tyr
500 505 510

Pro Gly Leu Leu Leu Glu Lys Cys His Pro Asn Ser Ile Phe Gly Glu
515 520 525

Ser Met Ile Glu Ile Gly Ala Pro Phe Ser Leu Lys Gly Leu Leu Gly
530 535 540

Asn Pro Ile Cys Ser Pro Glu Tyr Trp Lys Pro Ser Thr Phe Gly Gly
545 550 555 560

Glu Met Gly Phe Asn Met Val Lys Thr Ala Thr Leu Lys Lys Leu Val
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Cys Leu Asn Thr Lys Thr Cys Pro Tyr Val Ser Phe Arg Val Pro Asp
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Pro His Gln Asp Gly Gly Pro Gly Val Glu Arg Pro Ser Thr Glu Leu
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<211> 3632
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<213> Canis familiaris

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180

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240

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300

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Cys Pro Thr Pro Met Gly Val Lys Gly Lys Lys Glu Leu Pro Asp Ser
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Val Gly Gln Glu Val Phe Gly Leu Val Pro Gly Leu Met Met Tyr Ala
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Thr Ile Trp Leu Arg Glu His Asn Arg Val Cys Asp Val Leu Lys Gln
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Glu His Pro Glu Trp Asp Asp Glu Arg Leu Phe Gln Thr Ser Arg Leu
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Ile Leu Ile Gly Glu Thr Ile Lys Ile Val Ile Glu Asp Tyr Val Gln
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His Leu Ser Gly Tyr His Phe Lys Leu Lys Phe Asp Pro Glu Leu Leu
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Thr Leu Tyr His Trp His Pro Leu Leu Pro Asp Thr Leu Gln Ile Asp
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Cys Pro Phe Thr Ala Phe Ser Val Gln Asp Gly Gln Leu Thr Lys Thr
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Cys Gln His Gln Gly Ile Cys Val Arg Phe Gly Leu Asp Arg Tyr Gln
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Cys Asp Cys Thr Arg Thr Gly Tyr Ser Gly Pro Asn Cys Thr Ile Pro
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Gly Leu Trp Thr Trp Leu Arg Asn Ser Leu Arg Pro Ser Pro Ser Phe
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Thr His Phe Leu Leu Thr His Gly Arg Trp Phe Trp Glu Phe Val Asn
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Ala Thr Phe Ile Arg Glu Met Arg Met Arg Leu Val Leu Thr Val Arg
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Ser Asn Leu Ile Pro Ser Pro Pro Thr Tyr Asn Ser Ala His Asp Tyr
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Lys Gln Leu Pro Asp Ala Gln Leu Leu Ala Arg Arg Phe Leu Leu Arg
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Asp Gly Lys Leu Lys Tyr Gln Val Leu Asp Gly Glu Met Tyr Pro Pro
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Ser Val Glu Glu Ala Pro Val Leu Met His Tyr Pro Arg Gly Ile Pro
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Pro Gln Ser Gln Met Ala Val Gly Gln Glu Val Phe Gly Leu Leu Pro
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Cys Asp Leu Leu Lys Ala Glu His Pro Thr Trp Gly Asp Glu Gln Leu
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Ile Glu Glu Tyr Val Gln Gln Leu Ser Gly Tyr Phe Leu Gln Leu Lys
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Phe Asp Pro Glu Leu Leu Phe Gly Val Gln Phe Gln Tyr Arg Asn Arg
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Ile Ala Met Glu Phe Asn His Leu Tyr His Trp His Pro Leu Met Pro
385                 390                 395                 400

Asp Ser Phe Lys Val Gly Ser Gln Glu Tyr Ser Tyr Glu Gln Phe Leu
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Asp His His Ile Leu His Val Ala Val Asp Val Ile Arg Glu Ser Arg
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Glu Met Arg Leu Gln Pro Phe Asn Glu Tyr Arg Lys Arg Phe Gly Met
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Pro Gly Leu Leu Leu Glu Lys Cys His Pro Asn Ser Ile Phe Gly Glu
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3387

<210> 8
<211> 604
<212> PRT
<213> Homo sapiens

<400> 8

Met Leu Ala Arg Ala Leu Leu Leu Cys Ala Val Leu Ala Leu Ser His
1 5 10 15

Thr Ala Asn Pro Cys Cys Ser His Pro Cys Gln Asn Arg Gly Val Cys
20 25 30

Met Ser Val Gly Phe Asp Gln Tyr Lys Cys Asp Cys Thr Arg Thr Gly
35 40 45

Phe Tyr Gly Glu Asn Cys Ser Thr Pro Glu Phe Leu Thr Arg Ile Lys
50 55 60

Leu Phe Leu Lys Pro Thr Pro Asn Thr Val His Tyr Ile Leu Thr His
65 70 75 80

Phe Lys Gly Phe Trp Asn Val Val Asn Asn Ile Pro Phe Leu Arg Asn
85 90 95

Ala Ile Met Ser Tyr Val Leu Thr Ser Arg Ser His Leu Ile Asp Ser
100 105 110

Pro Pro Thr Tyr Asn Ala Asp Tyr Gly Tyr Lys Ser Trp Glu Ala Phe
115 120 125

Ser Asn Leu Ser Tyr Tyr Thr Arg Ala Leu Pro Pro Val Pro Asp Asp
130 135 140

Cys Pro Thr Pro Leu Gly Val Lys Gly Lys Lys Gln Leu Pro Asp Ser
145 150 155 160

Asn Glu Ile Val Glu Lys Leu Leu Arg Arg Lys Phe Ile Pro Asp
165 170 175

Pro Gln Gly Ser Asn Met Met Phe Ala Phe Phe Ala Gln His Phe Thr
180 185 190

His Gln Phe Phe Lys Thr Asp His Lys Arg Gly Pro Ala Phe Thr Asn
195 200 205

Gly Leu Gly His Gly Val Asp Leu Asn His Ile Tyr Gly Glu Thr Leu
210 215 220

Ala Arg Gln Arg Lys Leu Arg Leu Phe Lys Asp Gly Lys Met Lys Tyr
225 230 235 240

Gln Ile Ile Asp Gly Glu Met Tyr Pro Pro Thr Val Lys Asp Thr Gln
245 250 255

Ala Glu Met Ile Tyr Pro Pro Gln Val Pro Glu His Leu Arg Phe Ala
260 265 270

Val Gly Gln Glu Val Phe Gly Leu Val Pro Gly Leu Met Met Tyr Ala
275 280 285

Thr Ile Trp Leu Arg Glu His Asn Arg Val Cys Asp Val Leu Lys Gln
290 295 300

Glu His Pro Glu Trp Gly Asp Glu Gln Leu Phe Gln Thr Ser Arg Leu
305 310 315 320

Ile Leu Ile Gly Glu Thr Ile Lys Ile Val Ile Glu Asp Tyr Val Gln
325 330 335

His Leu Ser Gly Tyr His Phe Lys Leu Lys Phe Asp Pro Glu Leu Leu
340 345 350

Phe Asn Lys Gln Phe Gln Tyr Gln Asn Arg Ile Ala Ala Glu Phe Asn
355 360 365

Thr Leu Tyr His Trp His Pro Leu Leu Pro Asp Thr Phe Gln Ile His
370 375 380

Asp Gln Lys Tyr Asn Tyr Gln Gln Phe Ile Tyr Asn Asn Ser Ile Leu
385 390 395 400

Leu Glu His Gly Ile Thr Gln Phe Val Glu Ser Phe Thr Arg Gln Ile
405 410 415

Ala Gly Arg Val Ala Gly Gly Arg Asn Val Pro Pro Ala Val Gln Lys
420 425 430

Val Ser Gln Ala Ser Ile Asp Gln Ser Arg Gln Met Lys Tyr Gln Ser
435 440 445

Phe Asn Glu Tyr Arg Lys Arg Phe Met Leu Lys Pro Tyr Glu Ser Phe

```
    450                 455                 460

Glu Glu Leu Thr Gly Glu Lys Glu Met Ser Ala Glu Leu Glu Ala Leu
465                 470                 475                 480

Tyr Gly Asp Ile Asp Ala Val Glu Leu Tyr Pro Ala Leu Leu Val Glu
                485                 490                 495

Lys Pro Arg Pro Asp Ala Ile Phe Gly Glu Thr Met Val Glu Val Gly
            500                 505                 510

Ala Pro Phe Ser Leu Lys Gly Leu Met Gly Asn Val Ile Cys Ser Pro
        515                 520                 525

Ala Tyr Trp Lys Pro Ser Thr Phe Gly Gly Glu Val Gly Phe Gln Ile
    530                 535                 540

Ile Asn Thr Ala Ser Ile Gln Ser Leu Ile Cys Asn Asn Val Lys Gly
545                 550                 555                 560

Cys Pro Phe Thr Ser Phe Ser Val Pro Asp Pro Glu Leu Ile Lys Thr
                565                 570                 575

Val Thr Ile Asn Ala Ser Ser Ser Arg Ser Gly Leu Asp Asp Ile Asn
            580                 585                 590

Pro Thr Val Leu Leu Lys Glu Arg Ser Thr Glu Leu
        595                 600
```

<210> 9
<211> 604
<212> PRT
<213> Mus musculus

<400> 9

```
Met Leu Phe Arg Ala Val Leu Leu Cys Ala Ala Leu Gly Leu Ser Gln
  1               5                  10                  15

Ala Ala Asn Pro Cys Cys Ser Asn Pro Cys Gln Asn Arg Gly Glu Cys
             20                  25                  30

Met Ser Thr Gly Phe Asp Gln Tyr Lys Cys Asp Cys Thr Arg Thr Gly
         35                  40                  45

Phe Tyr Gly Glu Asn Cys Thr Thr Pro Glu Phe Leu Thr Arg Ile Lys
     50                  55                  60

Leu Leu Leu Lys Pro Thr Pro Asn Thr Val His Tyr Ile Leu Thr His
 65                  70                  75                  80

Phe Lys Gly Val Trp Asn Ile Val Asn Asn Ile Pro Phe Leu Arg Ser
                 85                  90                  95

Leu Thr Met Lys Tyr Val Leu Thr Ser Arg Ser Tyr Leu Ile Asp Ser
```

100 105 110

Pro Pro Thr Tyr Asn Val His Tyr Gly Tyr Lys Ser Trp Glu Ala Phe
115 120 125

Ser Asn Leu Ser Tyr Tyr Thr Arg Ala Leu Pro Pro Val Ala Asp Asp
130 135 140

Cys Pro Thr Pro Met Gly Val Lys Gly Asn Lys Glu Leu Pro Asp Ser
145 150 155 160

Lys Glu Val Leu Glu Lys Val Leu Leu Arg Arg Glu Phe Ile Pro Asp
165 170 175

Pro Gln Gly Ser Asn Met Met Phe Ala Phe Phe Ala Gln His Phe Thr
180 185 190

His Gln Phe Phe Lys Thr Asp His Lys Arg Gly Pro Gly Phe Thr Arg
195 200 205

Gly Leu Gly His Gly Val Asp Leu Asn His Ile Tyr Gly Glu Thr Leu
210 215 220

Asp Arg Gln His Lys Leu Arg Leu Phe Lys Asp Gly Lys Leu Lys Tyr
225 230 235 240

Gln Val Ile Gly Gly Glu Val Tyr Pro Pro Thr Val Lys Asp Thr Gln
245 250 255

Val Glu Met Ile Tyr Pro Pro His Ile Pro Glu Asn Leu Gln Phe Ala
260 265 270

Val Gly Gln Glu Val Phe Gly Leu Val Pro Gly Leu Met Met Tyr Ala
275 280 285

Thr Ile Trp Leu Arg Glu His Asn Arg Val Cys Asp Ile Leu Lys Gln
290 295 300

Glu His Pro Glu Trp Gly Asp Glu Gln Leu Phe Gln Thr Ser Arg Leu
305 310 315 320

Ile Leu Ile Gly Glu Thr Ile Lys Ile Val Ile Glu Asp Tyr Val Gln
325 330 335

His Leu Ser Gly Tyr His Phe Lys Leu Lys Phe Asp Pro Glu Leu Leu
340 345 350

Phe Asn Gln Gln Phe Gln Tyr Gln Asn Arg Ile Ala Ser Glu Phe Asn
355 360 365

Thr Leu Tyr His Trp His Pro Leu Leu Pro Asp Thr Phe Asn Ile Glu
370 375 380

Asp Gln Glu Tyr Ser Phe Lys Gln Phe Leu Tyr Asn Asn Ser Ile Leu
385 390 395 400

```
Leu Glu His Gly Leu Thr Gln Phe Val Glu Ser Phe Thr Arg Gln Ile
                405                 410                 415

Ala Gly Arg Val Ala Gly Gly Arg Asn Val Pro Ile Ala Val Gln Ala
            420                 425                 430

Val Ala Lys Ala Ser Ile Asp Gln Ser Arg Glu Met Lys Tyr Gln Ser
        435                 440                 445

Leu Asn Glu Tyr Arg Lys Arg Phe Ser Leu Lys Pro Tyr Thr Ser Phe
    450                 455                 460

Glu Glu Leu Thr Gly Glu Lys Glu Met Ala Ala Glu Leu Lys Ala Leu
465                 470                 475                 480

Tyr Ser Asp Ile Asp Val Met Glu Leu Tyr Pro Ala Leu Leu Val Glu
                485                 490                 495

Lys Pro Arg Pro Asp Ala Ile Phe Gly Glu Thr Met Val Glu Leu Gly
            500                 505                 510

Ala Pro Phe Ser Leu Lys Gly Leu Met Gly Asn Pro Ile Cys Ser Pro
        515                 520                 525

Gln Tyr Trp Lys Pro Ser Thr Phe Gly Gly Glu Val Gly Phe Lys Ile
    530                 535                 540

Ile Asn Thr Ala Ser Ile Gln Ser Leu Ile Cys Asn Asn Val Lys Gly
545                 550                 555                 560

Cys Pro Phe Thr Ser Phe Asn Val Gln Asp Pro Gln Pro Thr Lys Thr
                565                 570                 575

Ala Thr Ile Asn Ala Ser Ala Ser His Ser Arg Leu Asp Asp Ile Asn
            580                 585                 590

Pro Thr Val Leu Ile Lys Arg Arg Ser Thr Glu Leu
        595                 600
```

<210> 10
<211> 604
<212> PRT
<213> Rattus norvegicus

<400> 10

```
Met Leu Phe Arg Ala Val Leu Leu Cys Ala Ala Leu Ala Leu Ser His
  1               5                  10                  15

Ala Ala Asn Pro Cys Cys Ser Asn Pro Cys Gln Asn Arg Gly Glu Cys
             20                  25                  30

Met Ser Ile Gly Phe Asp Gln Tyr Lys Cys Asp Cys Thr Arg Thr Gly
         35                  40                  45
```

```
Phe Tyr Gly Glu Asn Cys Thr Thr Pro Glu Ile Leu Thr Arg Ile Lys
     50                  55                  60

Leu Leu Leu Lys Pro Thr Pro Asn Thr Val His Tyr Ile Leu Thr His
 65                  70                  75                  80

Phe Lys Gly Val Trp Asn Ile Val Asn Asn Ile Pro Phe Leu Arg Asn
                 85                  90                  95

Ser Ile Met Arg Tyr Val Leu Thr Ser Arg Ser His Leu Ile Asp Ser
            100                 105                 110

Pro Pro Thr Tyr Asn Val His Tyr Gly Tyr Lys Ser Trp Glu Ala Phe
        115                 120                 125

Ser Asn Leu Ser Tyr Tyr Thr Arg Ala Leu Pro Pro Val Ala Asp Asp
    130                 135                 140

Cys Pro Thr Pro Met Gly Val Lys Gly Asn Lys Glu Leu Pro Asp Ser
145                 150                 155                 160

Lys Glu Val Leu Glu Lys Val Leu Leu Arg Arg Glu Phe Ile Pro Asp
                165                 170                 175

Pro Gln Gly Thr Asn Met Met Phe Ala Phe Phe Ala Gln His Phe Thr
            180                 185                 190

His Gln Phe Phe Lys Thr Asp Gln Lys Arg Gly Pro Gly Phe Thr Arg
        195                 200                 205

Gly Leu Gly His Gly Val Asp Leu Asn His Val Tyr Gly Glu Thr Leu
    210                 215                 220

Asp Arg Gln His Lys Leu Arg Leu Phe Gln Asp Gly Lys Leu Lys Tyr
225                 230                 235                 240

Gln Val Ile Gly Gly Glu Val Tyr Pro Pro Thr Val Lys Asp Thr Gln
                245                 250                 255

Val Asp Met Ile Tyr Pro Pro His Val Pro Glu His Leu Arg Phe Ala
            260                 265                 270

Val Gly Gln Glu Val Phe Gly Leu Val Pro Gly Leu Met Met Tyr Ala
        275                 280                 285

Thr Ile Trp Leu Arg Glu His Asn Arg Val Cys Asp Ile Leu Lys Gln
    290                 295                 300

Glu His Pro Glu Trp Asp Asp Glu Arg Leu Phe Gln Thr Ser Arg Leu
305                 310                 315                 320

Ile Leu Ile Gly Glu Thr Ile Lys Ile Val Ile Glu Asp Tyr Val Gln
                325                 330                 335

His Leu Ser Gly Tyr His Phe Lys Leu Lys Phe Asp Pro Glu Leu Leu
            340                 345                 350
```

```
Phe Asn Gln Gln Phe Gln Tyr Gln Asn Arg Ile Ala Ser Glu Phe Asn
        355                 360                 365

Thr Leu Tyr His Trp His Pro Leu Leu Pro Asp Thr Phe Asn Ile Glu
    370                 375                 380

Asp Gln Glu Tyr Thr Phe Lys Gln Phe Leu Tyr Asn Asn Ser Ile Leu
385                 390                 395                 400

Leu Glu His Gly Leu Ala His Phe Val Glu Ser Phe Thr Arg Gln Ile
                405                 410                 415

Ala Gly Arg Val Ala Gly Gly Arg Asn Val Pro Ile Ala Val Gln Ala
            420                 425                 430

Val Ala Lys Ala Ser Ile Asp Gln Ser Arg Glu Met Lys Tyr Gln Ser
        435                 440                 445

Leu Asn Glu Tyr Arg Lys Arg Phe Ser Leu Lys Pro Tyr Thr Ser Phe
    450                 455                 460

Glu Glu Leu Thr Gly Glu Lys Glu Met Ala Ala Glu Leu Lys Ala Leu
465                 470                 475                 480

Tyr His Asp Ile Asp Ala Met Glu Leu Tyr Pro Ala Leu Leu Val Glu
                485                 490                 495

Lys Pro Arg Pro Asp Ala Ile Phe Gly Glu Thr Met Val Glu Leu Gly
            500                 505                 510

Ala Pro Phe Ser Leu Lys Gly Leu Met Gly Asn Pro Ile Cys Ser Pro
        515                 520                 525

Gln Tyr Trp Lys Pro Ser Thr Phe Gly Gly Glu Val Gly Phe Arg Ile
    530                 535                 540

Ile Asn Thr Ala Ser Ile Gln Ser Leu Ile Cys Asn Asn Val Lys Gly
545                 550                 555                 560

Cys Pro Phe Ala Ser Phe Asn Val Gln Asp Pro Gln Pro Thr Lys Thr
                565                 570                 575

Ala Thr Ile Asn Ala Ser Ala Ser His Ser Arg Leu Asp Asp Ile Asn
            580                 585                 590

Pro Thr Val Leu Ile Lys Arg Arg Ser Thr Glu Leu
        595                 600
```

<210> 11
<211> 604
<212> PRT
<213> Cavia porcellus

<400> 11

```
Met Leu Ala Arg Ala Leu Leu Leu Cys Ala Ala Leu Ala Leu Gly Gln
 1               5                  10                  15

Ala Ala Asn Pro Cys Cys Ser Asn Pro Cys Gln Asn Arg Gly Glu Cys
            20                  25                  30

Leu Ser Val Gly Phe Asp Arg Tyr Lys Cys Asp Cys Thr Arg Thr Gly
        35                  40                  45

Tyr Tyr Gly Glu Asn Cys Thr Thr Pro Glu Phe Leu Thr Arg Ile Lys
    50                  55                  60

Leu Leu Leu Lys Pro Thr Pro Asn Thr Val His Tyr Ile Leu Thr His
65                  70                  75                  80

Phe Lys Gly Val Trp Asn Ile Val Asn Asn Ile Pro Phe Leu Arg Asn
                85                  90                  95

Ala Ile Met Ile Tyr Val Leu Thr Ser Arg Ser His Leu Ile Asp Ser
            100                 105                 110

Pro Pro Thr Tyr Asn Ala His Tyr Gly Tyr Lys Ser Trp Glu Ala Phe
        115                 120                 125

Ser Asn Leu Ser Tyr Tyr Thr Arg Ala Leu Pro Pro Val Ala Asp Asp
    130                 135                 140

Cys Pro Thr Pro Met Gly Val Lys Gly Lys Lys Glu Leu Pro Asp Ser
145                 150                 155                 160

Asn Glu Val Leu Glu Lys Val Leu Leu Arg Arg Lys Phe Ile Pro Asp
                165                 170                 175

Pro Gln Gly Thr Asn Met Met Phe Ala Phe Phe Ala Gln His Phe Thr
            180                 185                 190

His Gln Phe Phe Lys Ser Asp Gln Lys Arg Gly Pro Ala Phe Thr Thr
        195                 200                 205

Gly Leu Ala His Gly Val Asp Leu Ser His Ile Tyr Gly Glu Thr Leu
    210                 215                 220

Asp Arg Gln His Lys Leu Arg Leu Phe Lys Asp Gly Lys Met Lys Tyr
225                 230                 235                 240

Gln Ile Ile Asp Gly Glu Met Tyr Pro Pro Thr Val Lys Glu Thr Gln
                245                 250                 255

Val Glu Met Met Tyr Pro Pro Tyr Ile Pro Glu His Ala Arg Phe Ala
            260                 265                 270

Val Gly Gln Glu Val Phe Gly Leu Val Pro Gly Leu Met Met Tyr Ala
        275                 280                 285

Thr Ile Trp Leu Arg Glu His Asn Arg Val Cys Asp Val Leu Lys Gln
```

```
    290                 295                 300

Glu His Pro Glu Trp Asp Asp Glu Arg Leu Phe Gln Thr Ser Arg Leu
305                 310                 315                 320

Ile Leu Ile Gly Glu Thr Ile Lys Ile Val Ile Glu Asp Tyr Val Gln
                325                 330                 335

His Leu Ser Gly Tyr His Phe Lys Leu Lys Phe Asp Pro Glu Leu Leu
            340                 345                 350

Phe Asn Gln Gln Phe Gln Tyr Gln Asn Arg Ile Ala Ser Glu Phe Asn
        355                 360                 365

Thr Leu Tyr His Trp His Pro Leu Leu Pro Asp Thr Phe Gln Ile Asp
    370                 375                 380

Asp Gln Val Tyr Asn Phe Gln Gln Phe Leu Tyr Asn Asn Ser Ile Leu
385                 390                 395                 400

Val Glu His Gly Leu Thr Gln Phe Val Glu Ser Phe Thr Lys Gln Ile
                405                 410                 415

Ala Gly Arg Val Ala Gly Gly Arg Asn Val Pro Leu Ala Val Gln Arg
            420                 425                 430

Val Ala Lys Ala Ser Ile Glu His Ser Arg Lys Met Lys Tyr Gln Ser
        435                 440                 445

Leu Asn Glu Tyr Arg Lys Arg Phe Leu Met Lys Pro Tyr Thr Ser Phe
    450                 455                 460

Glu Glu Leu Thr Gly Glu Lys Glu Met Ala Ala Gly Leu Glu Ala Leu
465                 470                 475                 480

Tyr Gly Asp Ile Asp Ala Met Glu Leu Tyr Pro Ala Leu Leu Val Glu
                485                 490                 495

Lys Pro Arg Pro Asp Ala Ile Phe Gly Glu Thr Met Val Glu Met Gly
            500                 505                 510

Ala Pro Phe Ser Leu Lys Gly Leu Met Gly Asn Pro Ile Cys Ser Pro
        515                 520                 525

His Tyr Trp Lys Pro Ser Thr Phe Gly Gly Glu Val Gly Phe Gln Ile
    530                 535                 540

Val Asn Thr Ala Ser Ile Gln Ser Leu Ile Cys Asn Asn Val Lys Gly
545                 550                 555                 560

Cys Pro Val Thr Ala Phe Asn Leu Pro Asp Pro Gln Leu Ala Lys Thr
                565                 570                 575

Val Thr Ile Asn Ala Ser Ala Ser His Ser Arg Leu Glu Asp Leu Ser
            580                 585                 590
```

Pro Thr Val Leu Leu Lys Gly Arg Ser Thr Glu Leu
595 600

<210> 12
<211> 604
<212> PRT
<213> Oryctolagus cuniculus

<400> 12

Met Leu Ala Arg Ala Leu Leu Leu Cys Ala Ala Val Ala Leu Ser His
1 5 10 15

Ala Ala Asn Pro Cys Cys Ser Asn Pro Cys Gln Asn Arg Gly Val Cys
20 25 30

Met Thr Met Gly Phe Asp Gln Tyr Lys Cys Asp Cys Thr Arg Thr Gly
35 40 45

Phe Tyr Gly Glu Asn Cys Ser Thr Pro Glu Phe Leu Thr Arg Ile Lys
50 55 60

Leu Leu Leu Lys Pro Thr Pro Asp Thr Val His Tyr Ile Leu Thr His
65 70 75 80

Phe Lys Gly Val Trp Asn Ile Val Asn Ser Ile Pro Phe Leu Arg Asn
85 90 95

Ser Ile Met Lys Tyr Val Leu Thr Ser Arg Ser His Met Ile Asp Ser
100 105 110

Pro Pro Thr Tyr Asn Val His Tyr Asn Tyr Lys Ser Trp Glu Ala Phe
115 120 125

Ser Asn Leu Ser Tyr Tyr Thr Arg Ala Leu Pro Pro Val Ala Asp Asp
130 135 140

Cys Pro Thr Pro Met Gly Val Lys Gly Lys Lys Glu Leu Pro Asp Ser
145 150 155 160

Lys Asp Val Val Glu Lys Leu Leu Leu Arg Arg Lys Phe Ile Pro Asp
165 170 175

Pro Gln Gly Thr Asn Met Met Phe Ala Phe Phe Ala Gln His Phe Thr
180 185 190

His Gln Phe Phe Lys Thr Asp Leu Lys Arg Gly Pro Ala Phe Thr Lys
195 200 205

Gly Leu Gly His Gly Val Asp Leu Asn His Ile Tyr Gly Glu Thr Leu
210 215 220

Asp Arg Gln His Lys Leu Arg Leu Phe Lys Asp Gly Lys Met Lys Tyr
225 230 235 240

```
Gln Val Ile Asp Gly Glu Val Tyr Pro Pro Thr Val Lys Asp Thr Gln
                245                 250                 255

Val Glu Met Ile Tyr Pro Pro His Ile Pro Ala His Leu Gln Phe Ala
            260                 265                 270

Val Gly Gln Glu Val Phe Gly Leu Val Pro Gly Leu Met Met Tyr Ala
        275                 280                 285

Thr Ile Trp Leu Arg Glu His Asn Arg Val Cys Asp Val Leu Lys Gln
    290                 295                 300

Glu His Pro Glu Trp Asp Asp Glu Gln Leu Phe Gln Thr Ser Arg Leu
305                 310                 315                 320

Ile Leu Ile Gly Glu Thr Ile Lys Ile Val Ile Glu Asp Tyr Val Gln
                325                 330                 335

His Leu Ser Gly Tyr His Phe Lys Leu Lys Phe Asp Pro Glu Leu Leu
            340                 345                 350

Phe Asn Gln Gln Phe Gln Tyr Gln Asn Arg Ile Ala Ala Glu Phe Asn
        355                 360                 365

Thr Leu Tyr His Trp His Pro Leu Leu Pro Asp Thr Phe Gln Ile Asp
    370                 375                 380

Asp Gln Gln Tyr Asn Tyr Gln Gln Phe Leu Tyr Asn Asn Ser Ile Leu
385                 390                 395                 400

Leu Glu His Gly Leu Thr Gln Phe Val Glu Ser Phe Thr Arg Gln Ile
                405                 410                 415

Ala Gly Arg Val Ala Gly Gly Arg Asn Val Pro Pro Ala Val Gln Lys
            420                 425                 430

Val Ala Lys Ala Ser Ile Asp Gln Ser Arg Gln Met Lys Tyr Gln Ser
        435                 440                 445

Leu Asn Glu Tyr Arg Lys Arg Phe Leu Leu Lys Pro Tyr Glu Ser Phe
    450                 455                 460

Glu Glu Leu Thr Gly Glu Lys Glu Met Ala Ala Glu Leu Glu Ala Leu
465                 470                 475                 480

Tyr Gly Asp Ile Asp Ala Val Glu Leu Tyr Pro Ala Leu Leu Val Glu
                485                 490                 495

Arg Pro Arg Pro Asp Ala Ile Phe Gly Glu Ser Met Val Glu Met Gly
            500                 505                 510

Ala Pro Phe Ser Leu Lys Gly Leu Met Gly Asn Pro Ile Cys Ser Pro
        515                 520                 525

Asn Tyr Trp Lys Pro Ser Thr Phe Gly Gly Glu Val Gly Phe Lys Ile
    530                 535                 540
```

```
Val Asn Thr Ala Ser Ile Gln Ser Leu Ile Cys Asn Asn Val Lys Gly
545                 550                 555                 560

Cys Pro Phe Thr Ser Phe Asn Val Pro Asp Pro Gln Leu Thr Lys Thr
                565                 570                 575

Val Thr Ile Asn Ala Ser Ala Ser His Ser Arg Leu Glu Asp Ile Asn
            580                 585                 590

Pro Thr Val Leu Leu Lys Gly Arg Ser Thr Glu Leu
        595                 600
```

<210> 13
<211> 604
<212> PRT
<213> Equus caballus

<400> 13

```
Met Leu Ala Arg Ala Leu Leu Leu Cys Val Ala Leu Ala Leu Gly His
  1               5                  10                  15

Ala Ala Asn Pro Cys Cys Ser Asn Pro Cys Gln Asn Arg Gly Val Cys
            20                  25                  30

Met Ser Val Gly Phe Asp Gln Tyr Gln Cys Asp Cys Thr Arg Thr Gly
        35                  40                  45

Phe Tyr Gly Glu Asn Cys Ser Thr Pro Glu Phe Leu Thr Arg Ile Lys
    50                  55                  60

Leu Phe Leu Lys Pro Thr Pro Asn Thr Val His Tyr Ile Leu Thr His
65                  70                  75                  80

Phe Lys Gly Val Trp Asn Ile Val Asn Ser Phe Pro Phe Leu Arg Asn
                85                  90                  95

Ala Val Met Lys Tyr Val Leu Val Ser Arg Ser His Leu Ile Glu Ser
            100                 105                 110

Pro Pro Thr Tyr Asn Ala Gln Tyr Gly Tyr Lys Ser Trp Glu Ser Phe
        115                 120                 125

Ser Asn Leu Ser Tyr Tyr Thr Arg Ala Leu Pro Pro Val Ala Asp Gly
    130                 135                 140

Cys Pro Thr Pro Met Gly Val Lys Gly Lys Lys Glu Leu Pro Asp Ser
145                 150                 155                 160

Lys Glu Ile Val Glu Lys Phe Leu Leu Arg Arg Lys Phe Ile Pro Asp
                165                 170                 175

Pro Gln Gly Thr Asn Met Met Phe Ala Phe Phe Ala Gln His Phe Thr
            180                 185                 190
```

```
His Gln Phe Phe Lys Thr Asp Pro Lys Arg Gly Pro Ala Phe Thr Lys
        195                 200                 205

Gly Leu Gly His Gly Val Asp Leu Ser His Ile Tyr Gly Glu Thr Leu
    210                 215                 220

Asp Arg Gln His Lys Leu Arg Leu Phe Lys Asp Gly Lys Met Lys Tyr
225                 230                 235                 240

Gln Ile Ile Asn Gly Glu Val Tyr Pro Pro Thr Val Lys Asp Thr Gln
                245                 250                 255

Val Glu Met Ile Tyr Pro Pro His Ile Pro Glu His Leu Arg Phe Ala
            260                 265                 270

Val Gly Gln Glu Val Phe Gly Leu Val Pro Gly Leu Met Met Tyr Ala
        275                 280                 285

Thr Ile Trp Leu Arg Glu His Asn Arg Val Cys Asp Val Leu Lys Gln
    290                 295                 300

Glu His Pro Glu Trp Asp Asp Glu Arg Leu Phe Gln Thr Ser Arg Leu
305                 310                 315                 320

Ile Leu Ile Gly Glu Thr Ile Lys Ile Val Ile Glu Asp Tyr Val Gln
                325                 330                 335

His Leu Ser Gly Tyr His Phe Lys Leu Lys Phe Asp Pro Glu Leu Leu
            340                 345                 350

Phe Asn Gln Gln Phe Gln Tyr Gln Asn Arg Ile Ala Ala Glu Phe Asn
        355                 360                 365

Thr Leu Tyr His Trp His Pro Leu Leu Pro Asp Thr Phe Gln Ile Asp
    370                 375                 380

Asp Gln Glu Tyr Asn Phe Gln Gln Phe Leu Tyr Asn Asn Ser Ile Leu
385                 390                 395                 400

Leu Glu His Gly Leu Thr Gln Phe Val Glu Ser Phe Ser Arg Gln Ile
                405                 410                 415

Ala Gly Arg Val Ala Gly Gly Arg Asn Val Pro Ala Ala Ala Gln Lys
            420                 425                 430

Ile Ala Lys Ala Ser Ile Asp Gln Ser Arg Glu Met Lys Tyr Gln Ser
        435                 440                 445

Leu Asn Glu Tyr Arg Lys Arg Phe Arg Leu Thr Pro Tyr Lys Ser Phe
    450                 455                 460

Glu Glu Leu Thr Gly Glu Lys Glu Met Ala Ala Glu Leu Glu Ala Leu
465                 470                 475                 480

Tyr Gly Asp Ile Asp Ala Met Glu Leu Tyr Pro Ala Leu Leu Val Glu
```

485 490 495

Lys Pro Arg Pro Asp Ala Ile Phe Gly Glu Thr Met Val Glu Leu Gly
500 505 510

Ala Pro Phe Ser Leu Lys Gly Leu Leu Gly Asn Pro Ile Cys Ser Pro
515 520 525

Asp Tyr Trp Lys Pro Ser Thr Phe Gly Gly Glu Val Gly Phe Lys Ile
530 535 540

Ile Asn Thr Ala Ser Ile Gln Ser Leu Ile Cys Asn Asn Val Lys Gly
545 550 555 560

Cys Pro Phe Thr Ala Phe Ser Val Gln Asp Pro Gln Leu Ser Lys Ala
565 570 575

Val Thr Ile Asn Ala Ser Ala Ser His Ser Gly Leu Asp Asp Val Asn
580 585 590

Pro Thr Val Leu Leu Lys Glu Arg Ser Thr Glu Leu
595 600

<210> 14
<211> 604
<212> PRT
<213> Ovis aries

<223> unsure at all Xaa locations
<400> 14

Met Leu Ala Arg Ala Leu Leu Leu Cys Ala Ala Val Val Cys Gly Xaa
1 5 10 15

Ala Ala Asn Pro Cys Cys Ser His Pro Cys Gln Asn Arg Gly Val Cys
20 25 30

Met Ser Val Gly Phe Asp Gln Tyr Lys Cys Asp Cys Thr Arg Thr Gly
35 40 45

Phe Tyr Gly Glu Asn Cys Thr Thr Pro Glu Phe Leu Thr Arg Ile Lys
50 55 60

Leu Leu Leu Lys Pro Thr Pro Asp Thr Val His Tyr Ile Leu Thr His
65 70 75 80

Phe Lys Gly Val Trp Asn Ile Val Asn Lys Ile Ser Phe Leu Arg Asn
85 90 95

Met Ile Met Arg Tyr Val Leu Thr Ser Arg Ser His Leu Ile Glu Ser
100 105 110

Pro Pro Thr Tyr Asn Val His Tyr Ser Tyr Lys Ser Trp Glu Ala Phe
115 120 125

```
Ser Asn Leu Ser Tyr Tyr Thr Arg Ala Leu Pro Pro Val Pro Asp Asp
    130                 135                 140

Cys Pro Thr Pro Met Gly Val Lys Gly Arg Lys Glu Leu Pro Asp Ser
145                 150                 155                 160

Lys Glu Val Val Lys Lys Val Leu Leu Arg Arg Lys Phe Ile Pro Asp
                165                 170                 175

Pro Gln Gly Thr Asn Leu Met Phe Ala Phe Phe Ala Gln His Phe Thr
            180                 185                 190

His Gln Phe Phe Lys Thr Asp Ile Glu Arg Gly Pro Ala Phe Thr Lys
        195                 200                 205

Gly Lys Asn His Gly Val Asp Leu Ser His Val Tyr Gly Glu Ser Leu
    210                 215                 220

Glu Arg Gln His Asn Arg Arg Leu Phe Lys Asp Gly Lys Met Lys Tyr
225                 230                 235                 240

Gln Met Ile Asn Gly Glu Met Tyr Pro Pro Thr Val Lys Asp Thr Gln
                245                 250                 255

Val Glu Met Ile Tyr Pro Pro His Ile Pro Glu His Leu Lys Phe Ala
            260                 265                 270

Val Gly Gln Glu Val Phe Gly Leu Val Pro Gly Leu Met Met Tyr Ala
        275                 280                 285

Thr Ile Trp Leu Arg Glu His Asn Arg Val Cys Asp Val Leu Lys Gln
    290                 295                 300

Glu His Pro Glu Trp Gly Asp Glu Gln Leu Phe Gln Thr Ser Arg Leu
305                 310                 315                 320

Ile Leu Ile Gly Glu Thr Ile Lys Ile Val Ile Glu Asp Tyr Val Gln
                325                 330                 335

His Leu Ser Gly Tyr His Phe Lys Leu Lys Phe Asp Pro Glu Leu Leu
            340                 345                 350

Phe Asn Gln Gln Phe Gln Tyr Gln Asn Arg Ile Ala Ala Glu Phe Asn
        355                 360                 365

Thr Leu Tyr His Trp His Pro Leu Leu Pro Asp Val Phe Gln Ile Asp
    370                 375                 380

Gly Gln Glu Tyr Asn Tyr Gln Gln Phe Ile Tyr Asn Asn Ser Val Leu
385                 390                 395                 400

Leu Glu His Gly Val Thr Gln Phe Val Glu Ser Phe Thr Arg Gln Ile
                405                 410                 415

Ala Gly Arg Val Ala Gly Arg Arg Asn Leu Pro Ala Ala Val Glu Lys
            420                 425                 430
```

```
Val Ser Lys Ala Ser Leu Asp Gln Ser Arg Glu Met Lys Tyr Gln Ser
        435                 440                 445

Phe Asn Glu Tyr Arg Lys Arg Phe Leu Leu Lys Pro Tyr Glu Ser Phe
    450                 455                 460

Glu Glu Leu Thr Gly Glu Lys Glu Met Ala Ala Glu Leu Glu Ala Leu
465                 470                 475                 480

Tyr Gly Asp Ile Asp Ala Met Glu Leu Tyr Pro Ala Leu Leu Val Glu
                485                 490                 495

Lys Pro Ala Pro Asp Ala Ile Phe Gly Glu Thr Met Val Glu Ala Gly
            500                 505                 510

Ala Pro Phe Ser Leu Lys Gly Leu Met Gly Asn Pro Ile Cys Ser Pro
        515                 520                 525

Glu Tyr Trp Lys Pro Ser Thr Phe Gly Gly Glu Val Gly Phe Lys Ile
    530                 535                 540

Ile Asn Thr Ala Ser Ile Gln Ser Leu Ile Cys Ser Asn Val Lys Gly
545                 550                 555                 560

Cys Pro Phe Thr Ser Phe Ser Val Gln Asp Ala His Leu Thr Lys Thr
                565                 570                 575

Val Thr Ile Asn Ala Ser Ser Ser His Ser Gly Leu Asp Asp Ile Asn
            580                 585                 590

Pro Thr Val Leu Leu Lys Glu Arg Ser Thr Glu Leu
        595                 600


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<211>      604
<212>      PRT
<213>      Gallus gallus

<223>      unsure at all Xaa locations
<400>      15

Met Leu Leu Pro Cys Ala Leu Leu Ala Ala Leu Leu Ala Ala Gly His
  1               5                  10                  15

Ala Ala Asn Pro Cys Cys Ser Leu Pro Cys Gln Asn Arg Gly Val Cys
            20                  25                  30

Met Thr Thr Gly Phe Asp Arg Tyr Glu Cys Asp Cys Thr Arg Thr Gly
        35                  40                  45

Tyr Tyr Gly Glu Asn Cys Thr Thr Pro Glu Phe Phe Thr Trp Leu Lys
    50                  55                  60

Leu Ile Leu Lys Pro Thr Pro Asn Thr Val His Tyr Ile Leu Thr His
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65 70 75 80

Phe Lys Gly Val Trp Asn Ile Ile Asn Asn Ile Ser Phe Leu Arg Asp
85 90 95

Thr Ile Met Arg Tyr Val Leu Thr Ser Arg Ser His Leu Ile Asp Ser
100 105 110

Pro Pro Thr Tyr Asn Ser Asp Tyr Ser Tyr Lys Ser Trp Glu Ala Tyr
115 120 125

Ser Asn Leu Ser Tyr Tyr Thr Arg Ser Leu Pro Pro Val Gly His Asp
130 135 140

Cys Pro Thr Pro Met Gly Val Lys Gly Lys Lys Glu Leu Pro Asp Ser
145 150 155 160

Lys Leu Ile Val Glu Lys Phe Leu Leu Arg Arg Lys Phe Ile Pro Asp
165 170 175

Pro Gln Gly Thr Asn Val Met Phe Thr Phe Phe Ala Gln His Phe Thr
180 185 190

His Gln Phe Phe Lys Thr Asp His Lys Lys Gly Pro Gly Phe Thr Lys
195 200 205

Ala Tyr Gly His Gly Val Asp Leu Asn His Ile Tyr Gly Glu Thr Leu
210 215 220

Glu Arg Gln Leu Lys Leu Arg Leu Arg Lys Asp Gly Lys Leu Lys Tyr
225 230 235 240

Gln Met Ile Asp Gly Glu Met Tyr Pro Pro Thr Val Lys Asp Thr Gln
245 250 255

Ala Glu Met Ile Tyr Pro Pro His Val Pro Glu His Leu Gln Phe Ser
260 265 270

Val Gly Gln Glu Val Phe Gly Leu Val Pro Gly Leu Met Met Tyr Ala
275 280 285

Thr Ile Trp Leu Arg Glu His Asn Arg Val Cys Asp Val Leu Lys Gln
290 295 300

Glu His Pro Glu Trp Asp Asp Glu Gln Leu Phe Gln Thr Thr Arg Leu
305 310 315 320

Ile Leu Ile Gly Glu Thr Ile Lys Ile Val Ile Glu Asp Tyr Val Gln
325 330 335

His Leu Ser Gly Tyr His Phe Lys Leu Lys Phe Asp Pro Glu Leu Leu
340 345 350

Phe Asn Gln Arg Phe Gln Tyr Gln Asn Arg Ile Ala Ala Glu Phe Asn
355 360 365

Thr Leu Tyr His Trp His Pro Leu Leu Pro Asp Thr Phe Gln Ile His
370 375 380

Asn Gln Glu Tyr Thr Phe Gln Gln Phe Leu Tyr Asn Asn Ser Ile Met
385 390 395 400

Leu Glu His Gly Leu Ser His Met Val Lys Ser Phe Ser Lys Gln Ser
405 410 415

Ala Gly Arg Val Ala Gly Gly Lys Asn Val Pro Ala Ala Val Gln Lys
420 425 430

Val Ala Lys Ala Ser Ile Asp Gln Ser Arg Gln Met Arg Tyr Gln Ser
435 440 445

Leu Asn Glu Tyr Arg Lys Arg Phe Met Leu Lys Pro Phe Lys Ser Phe
450 455 460

Glu Glu Leu Thr Gly Glu Lys Glu Met Ala Ala Glu Leu Glu Glu Leu
465 470 475 480

Tyr Gly Asp Ile Asp Ala Met Glu Leu Tyr Pro Gly Leu Leu Val Glu
485 490 495

Lys Pro Arg Pro Gly Ala Ile Phe Gly Glu Thr Met Val Glu Ile Gly
500 505 510

Ala Pro Phe Ser Leu Lys Gly Leu Met Gly Asn Thr Ile Cys Ser Pro
515 520 525

Glu Tyr Trp Lys Pro Ser Thr Phe Gly Gly Lys Val Gly Phe Glu Ile
530 535 540

Ile Asn Thr Ala Ser Leu Gln Lys Leu Ile Cys Asn Asn Val Lys Gly
545 550 555 560

Cys Pro Phe Thr Ala Phe His Val Leu Asn Pro Glu Pro Thr Xaa Glu
565 570 575

Ala Thr Ile Asn Val Ser Thr Ser Asn Thr Ala Met Glu Asp Ile Asn
580 585 590

Pro Thr Leu Leu Leu Lys Glu Gln Ser Ala Glu Leu
595 600

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/19565

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N9/02 C12Q1/26 C07K16/00 C12N5/10 C12Q1/68
G01N33/68 C12N15/62 A61K48/00 A61K38/43

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12Q C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EMBL, BIOSIS, MEDLINE, EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	COYNE D W ET AL: "REGULATION OF EICOSANOID BIOSYNTHESIS BY PHORBOL ESTER IN MADIN DARBY CANINE KIDNEY CELLS" AMERICAN JOURNAL OF PHYSIOLOGY, vol. 259, no. 4 PART 2, 1990, pages F698-F703, XP000942451 ISSN: 0002-9513 Abstract page F699, right-hand column, line 37 -page F699, right-hand column, line 56; figure 5 --- -/--	1-15,31, 32

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 December 2000	12.01.01

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Meyer, W

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BEVERELLI F ET AL: "Chronic inhibition of NO synthase enhances the production of prostacyclin in coronary arteries through upregulation of the cyclooxygenase type 1 isoform." FUNDAMENTAL & CLINICAL PHARMACOLOGY, vol. 11, no. 3, 1997, pages 252-259, XP000942441 ISSN: 0767-3981 abstract page 256, left-hand column, line 1 -page 256, left-hand column, line 13	1-15,31, 32
X	SMITH CAROLYN J ET AL: "Reduced gene expression of endothelial nitric oxide synthase and cyclooxygenase-1 in canine heart failure." CIRCULATION, vol. 90, no. 4 PART 2, 1994, page I299 XP000942524 67th Scientific Sessions of the American Heart Association;Dallas, Texas, USA; November 14-17, 1994 ISSN: 0009-7322 abstract	1-15,31, 32
A	THOMAS P S ET AL: "PROSTAGLANDIN D-2 PRODUCTION AND IDENTIFICATION OF PROSTAGLANDIN H SYNTHASE WITHIN CANINE MAST CELL GRANULE" AMERICAN JOURNAL OF PHYSIOLOGY, vol. 263, no. 2 PART 1, 1992, pages L168-L176, XP000942450 ISSN: 0002-9513 Abstract page L173, right-hand column, line 4 -page L173, right-hand column, line 14	1-15,31, 32
X	RICKETTS AP. ET AL.: "Evaluation of selective inhibition of canine cyclooxygenase 1 and 2 by carprofen and other nonsteroidal anti-inflammatory drugs 'see comments!" AM J VET RES 1998 NOV;59(11):1441-6, XP000942455 abstract page 1441, right-hand column, paragraph 2	1-15,31, 32
A	EP 0 736 769 A (MERCK FROSST CANADA INC) 9 October 1996 (1996-10-09) the whole document	1-15,31, 32
A	WO 94 06919 A (UNIV ROCHESTER) 31 March 1994 (1994-03-31) the whole document	1-15,31, 32

-/—

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	TAKAHASHI Y. ET AL.: "Immunoaffinity purification and cDNA cloning of human platelet prostaglandin endoperoxide synthase (cyclooxygenase)." BIOCHEM BIOPHYS RES COMMUN 1992 JAN 31;182(2):433-8, XP000941369 abstract; figure 1	1-15,31, 32
X	SCHIAFFINO M VITTORIA ET AL: "Cloning of a human homologue of the Xenopus laevis APX gene from the ocular albinism type 1 critical region." HUMAN MOLECULAR GENETICS, vol. 4, no. 3, 1995, pages 373-382, XP002155571 ISSN: 0964-6906	24,25, 27-30
Y	abstract	31-67
X	OSUKA KOJI ET AL: "Inducible cyclooxygenase expression in canine basilar artery after experimental subarachnoid hemorrhage." STROKE, vol. 29, no. 6, June 1998 (1998-06), pages 1219-1222, XP000942445 ISSN: 0039-2499	16-30
Y	discusssion abstract; figure 2	31-67
X	OSUKA KOJI ET AL: "Vasodilator effects on canine basilar artery induced by intracisternal interleukin-1beta." JOURNAL OF CEREBRAL BLOOD FLOW AND METABOLISM, vol. 17, no. 12, December 1997 (1997-12), pages 1337-1345, XP000942453 ISSN: 0271-678X	16-30
Y	abstract; figure 8	31-67
Y	WO 96 40720 A (UNIV ROCHESTER) 19 December 1996 (1996-12-19) claims 1-57	31-67
P,X	DATABASE EMBL 'Online! Accession No. AC046189, 14 April 2000 (2000-04-14) BIRREN B ET AL.: "Homo sapiens chromosome 4, clone RP11-356M17" XP002155572 abstract	16-23

-/--

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	HILDEBRAND J D ET AL: "SHROOM, A PDZ DOMAIN-CONTAINING ACTIN-BINDING PROTEIN, IS REQUIRED FOR NEURAL TUBE MORPHOGENESIS IN MICE" CELL,CELL PRESS, CAMBRIDGE, NA,US, vol. 99, no. 5, 24 November 1999 (1999-11-24), pages 485-497, XP000941350 ISSN: 0092-8674 abstract; figure 4	16-30

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 00/19565

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: -
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-15, 31 and 32

   Claims relating to Seq. Id. No. 1, 2, functional variants therof and the use thereof

2. Claims: 16-30 and 33-67

   Claims relating to Seq. Id. No. 3, 4, functional variants therof and the use thereof

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Present claims 24 and 25 relate to a protein defined by its corresponding DNA sequence. The corresponding sequence (SEQ ID: 4) is not a DNA sequence and thus the subject-matter of present claim is not clear within the meaning of Article 6 PCT.
However, the subject-matter of said claims has been interpreted as relating to a protein encoded by a nucleic acid sequence which specifically hybridizes to SEQ ID: 3 or its complement.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 00/19565

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0736769 A	09-10-1996	US 5543297 A	06-08-1996
		AT 155811 T	15-08-1997
		AU 5690694 A	19-07-1994
		CA 2151231 A	07-07-1994
		WO 9414977 A	07-07-1994
		DE 69312513 D	28-08-1997
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		ES 2105611 T	16-10-1997
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WO 9406919 A	31-03-1994	AT 179756 T	15-05-1999
		AU 688282 B	12-03-1998
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		DE 69324820 D	10-06-1999
		DE 69324820 T	13-01-2000
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		NZ 256776 A	24-10-1997
		US 5837479 A	17-11-1998
		US 6048850 A	11-04-2000
WO 9640720 A	19-12-1996	AU 6029396 A	30-12-1996
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		US 6048850 A	11-04-2000